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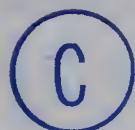


THE UNIVERSITY OF ALBERTA

TAXONOMY AND ECOLOGY OF DIPHYLLOBOTHRIUM

IN ALBERTA AND BRITISH COLUMBIA

by



DESMOND DARRINGTON ANTHONY

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES
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OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF ZOOLOGY

EDMONTON, ALBERTA

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UNIVERSITY OF ALBERTA
FACULTY OF GRADUATE STUDIES

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled Taxonomy and Ecology of Diphyllbothrium in Alberta and British Columbia submitted by Desmond Darrington Anthony in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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ABSTRACT

The Diphyllbothrium plerocercoids infecting certain species of fish from Kootenay Lake, British Columbia, and Iosegun Lake, Alberta, were investigated, and most of them identified.

A total of 1612 fish belonging to 14 species and 5 families were examined. Only five species, namely, kokanee (Oncorhynchus nerka), rainbow trout (Salmo gairdneri), and dolly varden char (Salvelinus malma) from Kootenay Lake, and northern pike (Esox lucius) and walleye (Stizostedion vitreum) from Iosegun Lake, were found infected.

Five species were identified from Kootenay Lake, namely, D. dendriticum, D. ditremum, D. osmeri, D. cordiceps, and D. latum, and a single species, D. latum, from Iosegun Lake. Two other plerocercoids infecting the three Kootenay Lake salmonids could not be identified to species.

Kokanee and dolly varden char were infected with all species except D. latum and D. cordiceps, but D. dendriticum was extremely rare. All species were found in trout, but D. latum only twice. D. ditremum was the most abundant species in Kootenay Lake, being found in high intensities in practically 100% of kokanee and all infected trout.

In order to facilitate identification of some of the plerocercoids and to determine potential definitive hosts, feeding experiments were conducted. Each type of plerocercoid was fed to

at least two of the four species of experimental animals used (dogs, cats, rats, and gulls).

The rate and pattern of growth of D. dendriticum, D. ditremum, D. osmeri, and D. cordiceps, were studied in some or all of these hosts, and observations were made on patency, apolysis, and longevity of each parasite species in each species of host.

A total of 222 birds of 15 species and 13 mammals were autopsied in attempt to discover the natural definitive host or hosts of the species occurring in fish. The mammals were all uninfected. The three species of gulls (Larus delawarensis, Larus argentatus, Larus californicus) and the one species of merganser examined, were the only birds infected, and D. ditremum and D. dendriticum were the only adult diphyllbothriids found in them. No definitive host was found for the other species.

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Thanks are also due to Messrs. Ted Rutherglen, Conservation Officer with the British Columbia Fish and Game Branch at Nelson, B.C.; Don McCulloch, former Conservation Officer with that department; "Skipper" Wilson; and Gil Palmer, all of whom assisted in no small way with fish collections in 1963 and 1964, and Mr. Ted Hunter who supplied the otters.

Messrs. Fred Jones and Arnold Cummings, resort operators on Kootenay Lake were exceptionally helpful throughout the study in securing for me viscera of, and data on, fish which would not otherwise have been available. I wish to thank them sincerely for their co-operation.

Mr. Larry Belway, now Supervisor of the Kootenay Trout Hatchery, and his staff, built the kennels at Nelson for housing

the dogs, and in many other ways contributed to this project. The staff of the Medical Vivarium, University of Alberta, cared for the dogs, cats, and wolves used in the experiments. I wish to thank them, and also some of my former colleagues from the Parasitology Division of the Department of Zoology, namely, Messrs. Mike Denny, Dick Gallimore and Lane Graham, for assisting in fish collections from Iosegun Lake in 1965, and especially Bill Turner who assisted with the project in general during the year.

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This list of acknowledgements makes it clear that only the responsibility to conduct the project was mine, but the project itself was a partnership which many shared.

The project was supported mainly by NRC operating grant No. A1464 (Dr. J.C. Holmes) and by Teaching Assistantship appointments.

Diphyllbothrium cordiceps (Leidy, 1872) Megitt, 1924

Fully relaxed 25-day specimen experimentally reared
in a dog; 392 cm. (13 ft.) long.

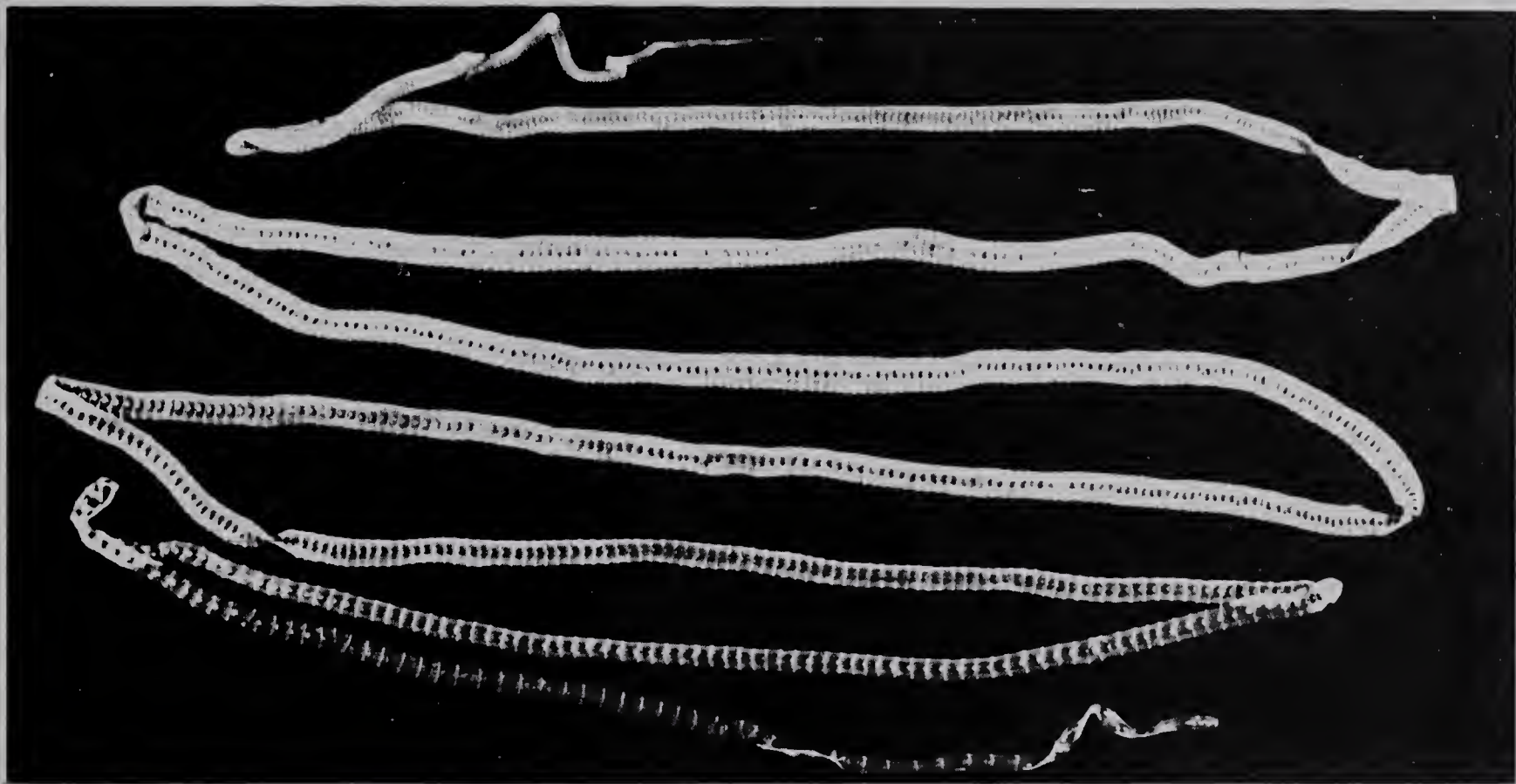


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INTRODUCTION

Interest was aroused in the diphyllbothriids of Kootenay Lake as a result of repeated reports of "worms in the flesh" of large rainbow trout (Salmo gairdneri) caught in the lake. Because large numbers of visitors are attracted to the area throughout the year, especially for sport fishing, the British Columbia Fish and Game Branch became concerned about these reports, and decided to investigate them.

In 1961, Harold Harvey, then of the Institute of Fisheries, University of British Columbia, identified the plerocercoids as Diphyllbothrium sp., and suggested that the large rainbow trout were only incidentally involved in the parasite's life-cycle, which normally might be completed through a species of fish more readily available to the definitive host. He therefore examined a sample of 110 kokanee (Oncorhynchus nerka) obtained between August 24 and 28 that year from the Meadow Creek spawning ground. Ninety-nine of these (90%) had plerocercoids encysted in the region of the stomach and mesenteries. Eight fish contained one or more large plerocercoids in the musculature as well. In order to ascertain the definitive host, ten gulls and one bear were examined. Diphyllbothrium sp. were found in eight of these gulls, shot in October, 1961 in the vicinity of two kokanee spawning areas. Tapeworm scolices were reported from among the villi

of the bear's intestine, but their identity was not established (Harvey, 1961).

Harvey did not positively identify any of the species involved, but stated that, except for some of the plerocercoids obtained from the musculature, the plerocercoids did not resemble those of D. latum, with which he was familiar. He further suggested subsequent life-cycle studies, to be aimed at rearing adult worms, since it was not possible at that time to identify species of Diphylllobothrium on the basis of larval characteristics alone.

The Department of Zoology, University of Alberta, became interested in this project through Mr. S.B. Smith, then a graduate student in the department, and Divisional Biologist with the British Columbia Fish and Game Branch. Through his co-operation and that of the Fish and Game Branch, the project was started on Kootenay Lake in May, 1963. Trout Lake, which is connected to Kootenay Lake by the Lardeau River, was included in August, 1964 when a heavily parasitized 8-lb rainbow trout caught on that lake was sent to the author.

Interest in Iosegun Lake, Alberta, dates back to the mid - 1950's when walleye (Stizostedion vitreum) and northern pike (Esox lucius) from the lake were reported to be parasitized by a tissue-invading worm which was probably the plerocercoid of Diphylllobothrium sp. In 1957, G.F. Hartman, Fisheries Biologist

with the Alberta Department of Lands and Forests, carried out a survey which revealed that 74.3% of the 117 walleye pike examined, 48.6% of the 31 northern pike, and 5.5% of the 18 perch, were carrying plerocercoids in the flesh (Hartman, 1957). Some of these plerocercoids were sent to the Institute of Parasitology, McDonald College, McGill University, where they were identified as "probably plerocercoids of Schistocephalus Creplin, 1829". It was thought "highly improbable that they were Diphyllbothrium". Dr. R.B. Miller of the Zoology Department, University of Alberta, later identified the worms as Diphyllbothrium sp. In 1960 and 1961, Dr. J.C. Holmes, Parasitologist with the Department of Zoology, carried out a survey which confirmed the identity of the worm. He also identified as "Diphyllbothrium sp. - probably latum", a mature strobila obtained in 1957 from a dog owned by a trapper who lives near the lake.

It is generally acknowledged that the species of Diphyllbothrium are rather difficult to identify, partly because of the nature of the characteristics used in distinguishing between them (Rausch, 1954; Vik, 1964a; Stunkard, 1965; Meyer, 1966).

Because the range of variability of these characteristics is poorly understood, particularly in cases in which a single species is capable of infecting a wide range of hosts, worms which showed some variations from standard descriptions of existing species frequently have been reported as new species, especially if they were reported from a new host. As a result,

a large number of species has been described for this genus, which Wardle et al. (1947) termed "a cumbersome group of about 70 species - many of them of dubious validity". Several additional species have been described since this statement was made; other characteristics of "taxonomic significance" have been introduced; and the problem of correctly identifying the members of this group has increased proportionately.

The value of larval characteristics in the systematics of the genus were apparently overlooked until the work of Kuhlman (1953a). Consequently, the useful literature on plerocercoids is rather sparse, comprising detailed descriptions of plerocercoids of Diphyllbothrium latum (L), Diphyllbothrium dendriticum (Nitzsch, 1824), Diphyllbothrium osmeri (von Linstow, 1878), Diphyllbothrium vogeli Kuhlman, 1953a (all described by Kuhlman, 1953a); Diphyllbothrium ursi Rausch, 1954; Diphyllbothrium dalliae Rausch, 1956; Diphyllbothrium norvegicum Vik, 1957; and Diphyllbothrium sebage (Ward, 1910) (described by Meyer and Robinson 1963). To this list may be added Diphyllbothrium cordiceps (Leidy, 1872) (partially described by Linton, 1891a).

The general life-cycle of Diphyllbothrium sp. (Fig. 1) is complex and is known to include hosts from different classes of animals. Piscivorous and omnivorous birds and mammals harbour the adult worm; various species of fish host the plerocercoid or third stage larva; and some species of copepodid crustaceans serve as host to the procercoid or second stage larva which develops from the ingested first stage larva or

coracidium.

In the present study an attempt is made to

(i) identify the species of Diphyllobothrium occurring in the study areas, with special reference to the plerocercoids found in fish;

(ii) discover the important ecological factors in their propagation; that is, to elucidate the life-cycle by ascertaining the actual and/or potential hosts at each stage of development; and

(iii) to learn something about the general biology of the worms.

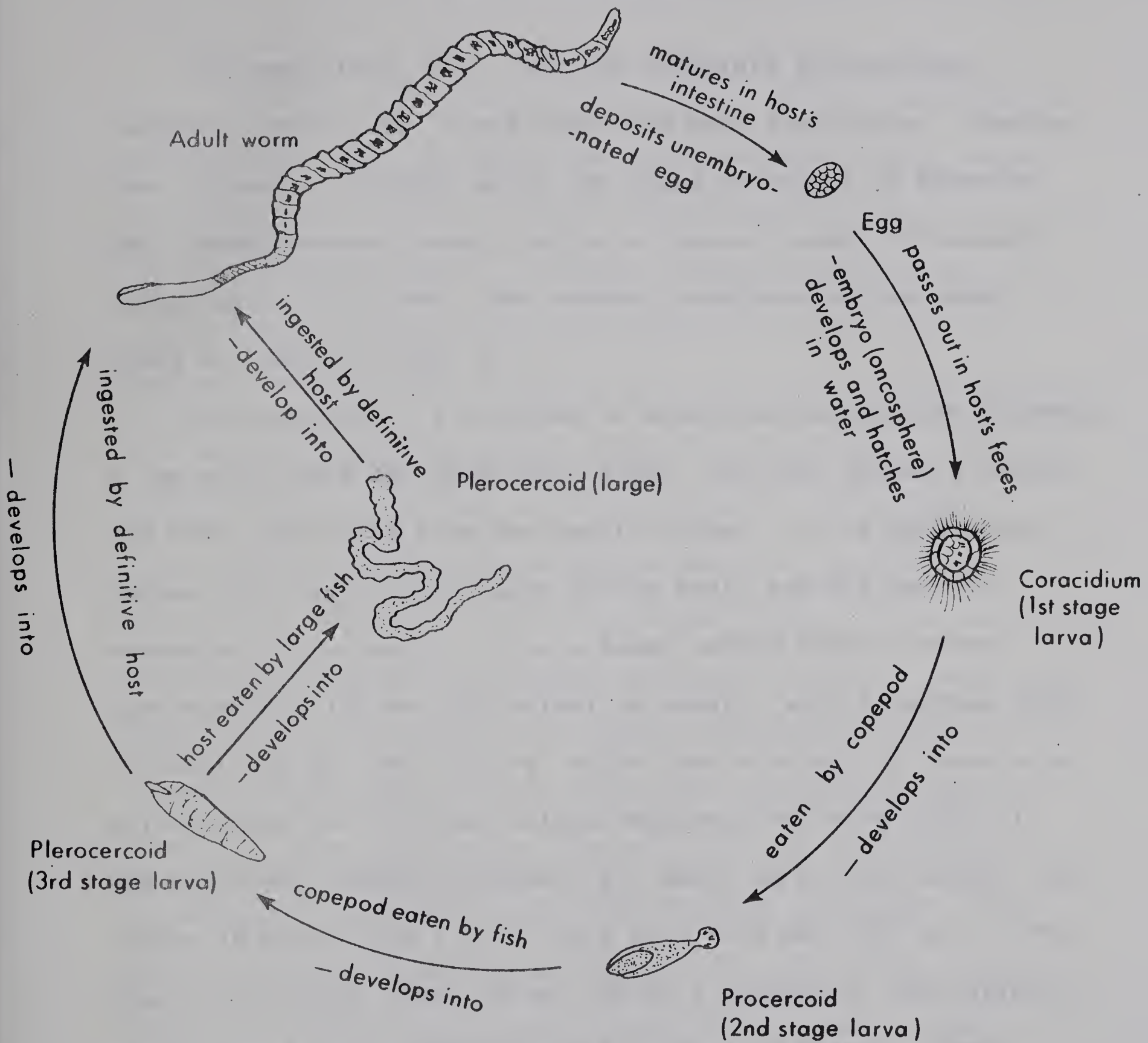


FIG. 1 — TYPICAL LIFE-CYCLE OF DIPHYLLOBOTHRIUM SP.

THE STUDY AREAS

Kootenay Lake, B.C., and the immediate surroundings, including Trout Lake, constituted the main study area. Iosegun Lake, Alberta, situated about 180 miles northwest of Edmonton, was a supplementary area in which a limited amount of investigation was carried out. The general locations of the study areas are shown in Fig. 2.

Kootenay Lake is situated in south-central British Columbia, a few miles from the Washington State - British Columbia border, and about 500 miles from the Pacific coast. It is sandwiched between the Purcell Mountains to the east, and the Selkirk Mountains to the west. It is a long, narrow body of water, approximately 110 km. (70 miles) in length, with a surface area of about 400 sq. km. (155 sq. miles), and consists of three arms which spread out from the Balfour-Kootenay Bay area (Fig. 3). The North Arm extends northward for about 64 km. (40 miles), and varies in width from 1.2 km. (3/4 mi.) to 4 km. (2.5 mi.). The South Arm extends about 48 km. (30 mi.) southward, and varies in width from 3.2 km. (2 miles) to 5.5 km. (3.5 miles). These two arms constitute the main lake. The West Arm, with its narrows and fast currents which give it the appearance more of a river than a lake, varies in width from about 0.5 km. (550 yards) at Fraser Narrows to 1.4 km. (0.9 miles) in the area between Harrop and Sunshine Bay. It extends west and south for

35 km. (22 miles) to drain into the Kootenay River, a major tributary of the Columbia River.

Numerous small precipitous streams and two medium-sized rivers empty into the lake. The Upper Kootenay River enters the South Arm, and the combined Lardeau-Duncan River enters the system at its north end. Because of its deep basin and steep, rocky shores, unsuited for the support of rooted aquatic vegetation, (Fig. 4a), Kootenay Lake must be characterized as oligotrophic. Depths ranging consistently between 100 and 150 metres (300 to 500 feet) are encountered in the middle.

However, for an oligotrophic lake, it is quite productive of several species of fish. Cartwright (1961) listed twenty-two species of fish "known, or suspected to be present" in the lake. Among these are rainbow trout (Salmo gairdneri), kokanee (Oncorhynchus nerka), dolly varden char (Salvelinus malma), burbot or ling (Lota lota), mountain white fish (Prosopium williamsoni), largemouth bass (Micropterus salmoides), and white sturgeon (Acipenser transmontanus). The first three of these form the basis of sport fishing on the lake. Catches of rainbow trout 20 to 25 pounds (10 - 11.5 kg.) in weight are not uncommon. These three salmonids use the streams tributary to the lake as spawning grounds. The upper reaches of the Lardeau River in the vicinity of Gerrard constitute the main spawning ground of rainbow trout. The lower Duncan River and some

streams along the West and South Arms are used to a much lesser extent. Spawning occurs from about the middle of April to about the end of May. Dolly varden spawn up some North Arm streams, such as the Duncan River, Meadow Creek, and Fry Creek, from late summer to early fall. Kokanee spawning occurs up several streams on all arms of the lake during late summer and early fall. Meadow Creek is the main spawning ground of the North Arm. Here, at the peak of the spawning activity, literally thousands of kokanee can be seen on the spawning bed. Akokli Creek and Sanca Creek are the main South Arm spawning beds; and Redfish Creek and Kokanee Creek are the main West Arm sites (Fig. 3).

The plankton fauna, although not abundant in numbers of species, is significant in quantity. Zyblut (unpublished data) identified three species of copepods prevalent throughout the lake: Diaptomus ashlandi, Cyclops bicuspidatus, and Epischura nevadensis. Diaptomus kenai have also been found, but these were extremely rare. The cladocerans identified were Daphnia thorata, Diaphnosoma sp., and Bosmina sp. Together with Mysis mysis, introduced from Waterton Lakes, Alberta, in 1949, these constitute the main source of food for small plankton-feeding fishes such as kokanee. Mayfly larvae and other aquatic insects make up the neuston.

Fish-eating mammals found in the immediate environment

of the lake include black bear (Ursus americanus), cougar (Felis concolor), lynx (Lynx canadensis), bob-cat (Lynx rufus), otter (Lutra canadensis), mink (Mustela vison), and coyote (Canis latrans). The piscivorous and omnivorous birds include three species of gulls: ringbilled gull (Larus delawarensis), California gull (Larus californicus), and herring gull (Larus argentatus); common mergansers (Mergus americanus); American coot (Fulica americana); horned grebe (Podiceps auritus), eared grebe (Podiceps caspicus), rednecked grebe (Podiceps grisegena), western grebe (Aechmophorus occidentalis); osprey (Pandion haliaetus); the common crow (Corvus brachyrhynchos); several species of ducks, and several other species of smaller water birds.

Many resort sites, several small to fair-sized communities, and one town of 10,000 population, Nelson, the administrative and commercial centre of that part of British Columbia, are located along the lake. The Nelson city sewer system discharges into the West Arm, and open garbage dumps are found at Nelson, Kaslo, Balfour, and at other points. Fishing and hunting are popular pastimes of many of the residents.

Trout Lake (Fig. 3) is much smaller than Kootenay Lake. It is approximately 24 km. (15 miles) long, and varies in width from 0.4 km. ($\frac{1}{4}$ mile) in the vicinity of American Point at the south end, to 1.6 km. (1 mile) at the north end.

The physical features of the surrounding region, climatic

conditions, the fish, avian and mammalian faunas of the area, as well as the morphometry of the lake, are very similar to those of the northern part of Kootenay Lake. Unlike the latter, however, Trout Lake is completely frozen over from January to the end of April every year.

Many small creeks and streams, several of which are of glacial origin, drain into the lake. Lardeau River, which is the connecting link between this lake and Kootenay Lake, leaves the former at its southeastern corner.

Apart from a few summer cottages and two hermit's cabins, the only settlement on this lake is Trout Lake City, a small resort and logging community at its north end. Gerrard, once a thriving centre, is now an almost completely deserted landmark at the south end of the lake.

Iosegun Lake (Fig. 2) is situated in the Swan Hills district of west central Alberta, about 180 miles northwest of Edmonton. It is a small mesotrophic lake 19 km. (12 miles) long and 6.5 km. (4 miles) wide at its widest point. According to Hartman (1957), "the basin is quite regular and slopes off to 40 ft. in a small area near the centre, [and] the north end...is shallow and weedy" (Fig. 4b).

The most abundant fish species found in the lake are northern pike (Esox lucius), walleye (Stizostedion vitreum), and perch (Perca flavescens). Sticklebacks (Gasterosteus sp.),

whitefish (Coregonus sp.), and tulibee (Leucichthyes sp.) are also found in large numbers. With regards to the planktonic fauna, Hartman (1957) found that Cyclops sp., Diaptomus sp., and Daphnia sp. were the common zooplankton.

The avifauna, which is especially abundant during the summer and autumn months, includes common loons (Gavia immer), several species of ducks including lesser scaup (Aythya affinis), white-winged scoter (Melanitta deglandi), canvas-back (Aythya valisineria), and mallard (Anas platyrhynchos); common mergansers (Mergus americanus); western grebes (Aechmophorus occidentalis); red-necked grebes (Podiceps grisegena); eared grebes (Podiceps caspicus); Franklin's gull (Larus pipixcan) and a few other species of gulls; common crows (Corvus brachyrhynchos), and ravens (Corvus corax).

Among the fish-eating mammals recorded in the area are black bears (Ursus americanus), grizzly bears (Ursus arctos horribilis), timber wolves (Canis lupus), coyotes (Canis latrans), lynx (Lynx canadensis), and mink (Mustela vison).

Except for a trapper's cabin, there is no permanent settlement on the banks of the lake; but certain areas are used for recreation and as camp sites during the warmer months of the year when a fair amount of sport fishing occurs on the lake. The only settlements of any size in the immediate vicinity are Fox Creek, an oil drilling camp eight miles from

the lake, and another oil trailer town about 5 miles away.



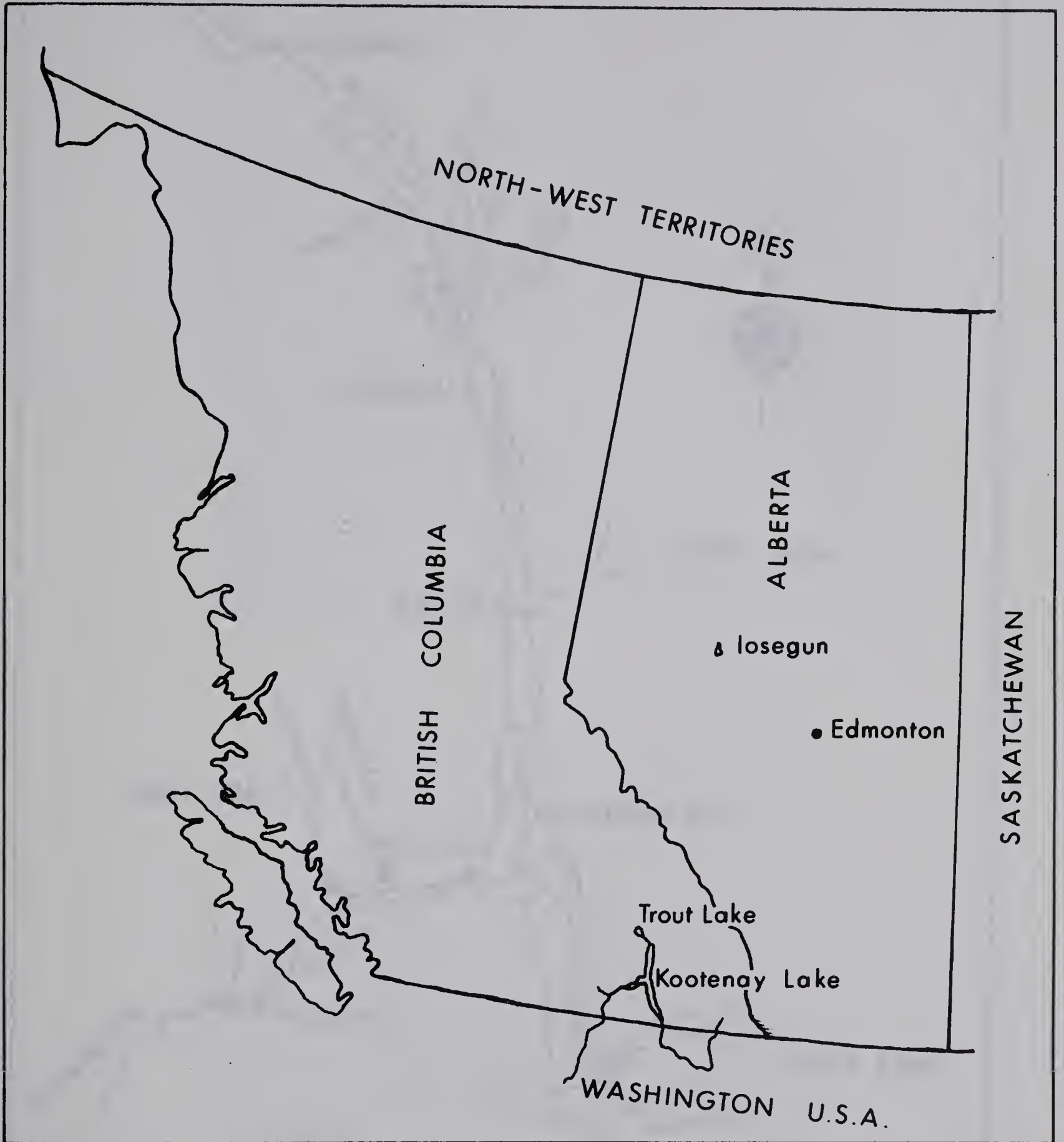


FIG. 2 — MAP SHOWING THE GENERAL LOCATIONS OF THE STUDY AREAS

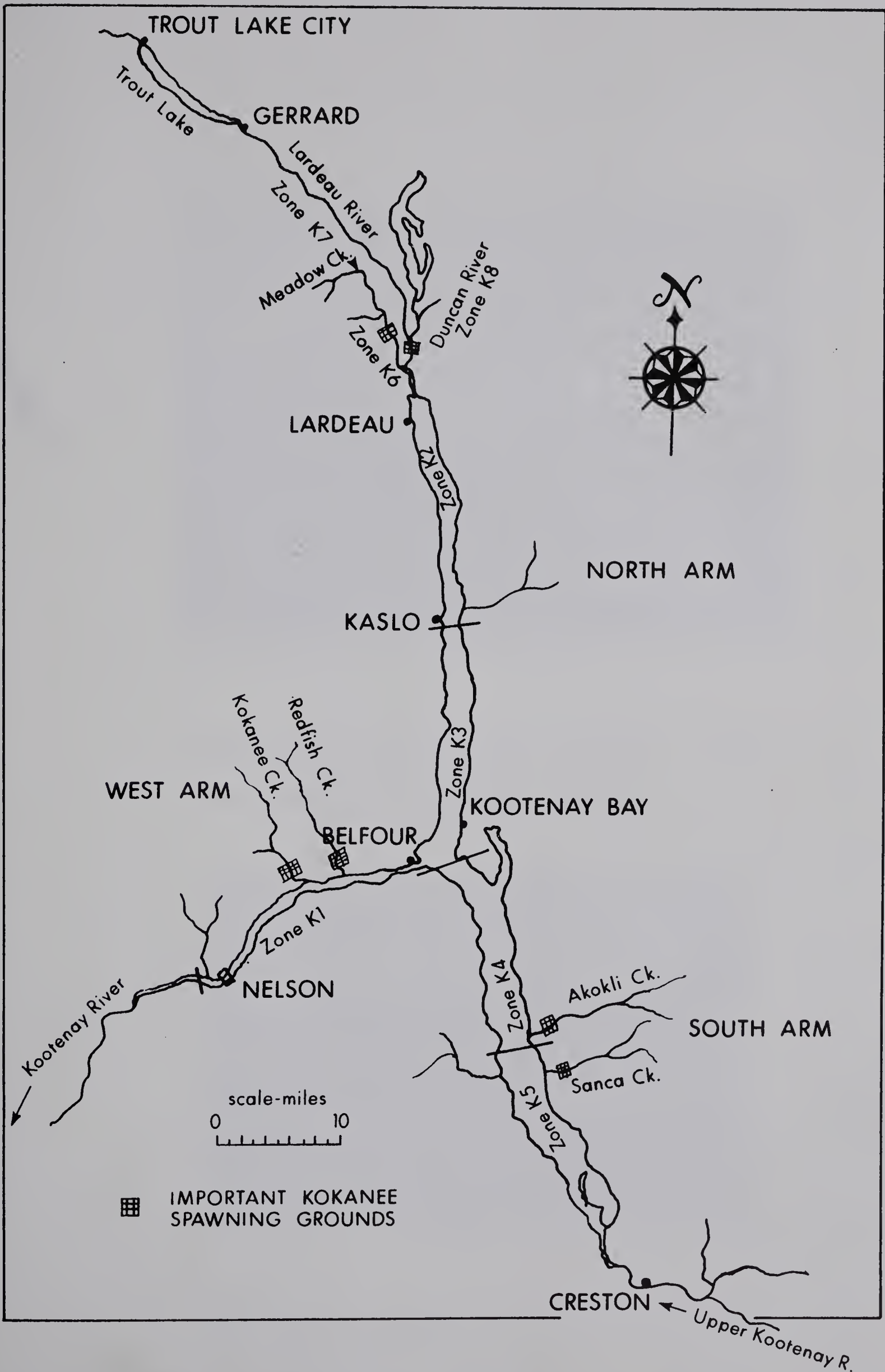


FIG. 3 - MAP OF KOOTENAY LAKE, B.C.

Fig. 4 (above) Portion of Kootenay Lake
showing steep shore-line.
(below) Portion of Iosegun Lake
showing weedy shore-line.



MATERIALS AND METHODS

A combination of field investigations into natural populations and laboratory studies, which included experimental life-cycle studies and histological procedures, has been utilized.

(1) - Field investigations

The material studied in these investigations included samples of:

(i) 1612 fish belonging to 14 species, but mainly kokanee, rainbow trout, and dolly varden char from Kootenay and Trout Lakes; and walleye and northern pike from Iosegun Lake;

(ii) 222 piscivorous and scavenger birds from 15 species but mainly three species of gulls, collected almost entirely from the Kootenay Lake area (6 specimens from Iosegun Lake);

(iii) 13 mammals obtained from the vicinity of Kootenay Lake; and

(iv) several thousand copepods from Kootenay Lake.

Samples were collected from Kootenay Lake from June to October 1963, from January to October in 1964 and 1965, and in March and from July to September 1966. About half of the kokanee examined were spawning fish collected from their spawning beds with dip-nets. Approximately 25% were donated by anglers

and resort operators, and the rest were obtained by gill-netting. All trout and dolly varden char examined were in the non-breeding state. About 15% of the trout were gill-netted, the remainder were obtained from anglers. These included some large specimens, only the viscera of which were available for examination. The rest of the fish was retained by the angler, but its length, weight, and sex were recorded, and scale samples were saved. All of the dolly varden char were obtained from anglers, except four which were caught in a fish trap.

Eight walleye and three pike were obtained from Iosegun Lake by angling in June 1966; the remainder were collected in June 1965 by angling and gill-netting.

All specimens were kept in cold-storage pending examination which was usually done within 8 hours of collection. Those which could not be examined within 12 hours, were frozen. At autopsy, the length, weight, and sex of each specimen were recorded, and scale samples from the region of the dorsal fin were taken for subsequent age determination. Because of their very small scales, and extensive regeneration of scales, dolly varden char were not aged.

The entire viscera of each specimen were examined for plerocercoids. Except in cases in which only the viscera was obtained, the musculature was examined by filletting the fish, holding the fillet in front of a bright light, and removing

worms seen in it, then cutting the fillets into thin slices. Pepsin digestion of a few kokanee stomachs after the method of Vik (1964b) yielded a few small plerocercoids which had escaped notice, but this digestion technique was not routinely used. The number of plerocercoids per fish, the host organs from which these were obtained, and whether they were encysted or unencysted, were recorded. Most of the worms (including all of those recovered in 1965 and 1966) were later identified to species.

Plerocercoids which were to be used in feeding experiments were kept in a cool place in covered dishes during the interval between removal from the fish and feeding to the experimental hosts (not usually longer than 15-20 minutes). Those intended for subsequent histological studies were washed in cold tap water, and were immediately fixed, or were allowed to relax and die in the water, before fixing in AFA (Cable, 1951), Bouin's fluid, Sanfelice solution, 10% neutralized formalin, or the alcohol-neutralized formalin solution of Wikgren (1964) - 1 part 10% neutralized-formalin to 10 parts 70% alcohol.

The birds were shot, then kept cool or frozen until examination. The mammals were obtained from the B.C. Fish and Game Branch or from a trapper operating north of Kootenay Lake, and were frozen until examination. Only the intestines of these animals were examined. Adult worms recovered were allowed to

relax and die in cold water in a refrigerator. The length of each worm, the size of its scolex, and the dimensions of early mature proglottids and of gravid proglottids from the middle and posterior portions of the strobila, were recorded, and the worms fixed in A.F.A.

Copepods were mounted in a drop of water, glycerine, or xylene on a clean slide, then examined under the microscope.

(2) - Experimental laboratory studies

During the period of study, a series of experimental studies, in which plerocercoids of each species were fed to dogs, cats, rats, and young ring-billed gulls, was carried out. Plerocercoids of D. dendriticum and D. ditremum were also fed to two timber wolves and to eight human volunteers.

The 59 dogs used were obtained from three sources:

(a) The Department of Animal Husbandry, University of British Columbia (12 laboratory-raised Labrador pups, 5-6 weeks old);

(b) The Medical Vivarium, University of Alberta (26 dogs of various breeds, mainly Labradors); and

(c) The city pound of Nelson, B.C. (21 dogs, mostly mongrels). The animals obtained from the pound were treated with Nemural (Winthrop Ltd., Toronto, Ontario), an anthelmintic drug which is effectual against tapeworms. Dogs from the vivarium were routinely dewormed by the staff, and were known

to be free of tapeworms; and the laboratory-raised dogs had never been exposed to tapeworms of any kind.

Seventeen of the 36 cats used were supplied by the vivarium where they were routinely dewormed. The others were bought from the Nelson city pound, and were also treated with Nemural. The white rats were taken from the stock maintained by the Department of Zoology, University of Alberta, were 2-12 months old, and had not been previously exposed to tapeworms. The two timber wolves were obtained from the vivarium, and were known to be free of tapeworm infection.

The gulls used in 1963 were raised from eggs collected from nesting grounds on Miquelon Lake, Alberta, incubated and hatched in an electric incubator. In 1964, both eggs and chicks were obtained from the Miquelon Lake nesting site, and in 1965 and 1966, chicks were collected from nesting sites on Beaverhill Lake, Alberta. Stool examinations were done on all the captured chicks in order to ensure that they were not previously infected with Diphylllobothrium. They ranged in age from 3 weeks to 4 months.

The dogs and the wolves were fed meat-based Tops and Rover dog foods. The cats were fed Purina cat biscuits, the rats Purina chow, and the gulls were fed minced meat, beef liver, and fish which had been deep-frozen and was known to be Diphylllobothrium-free.

At the university, the dogs, cats, and wolves were housed at the University Vivarium where they were looked after by the staff. The rats and gulls were kept in the animal room at the Zoology Department. In Nelson, B.C. where most of the experiments were carried out, three old concrete fish-ponds, about 4 feet deep, 20-25 feet long and about 6 feet wide, enclosed by a 7-foot high wire-mesh fence, were used as a day-run for the dogs. The gate of this enclosure was kept locked whenever the dogs were in there. At night, the animals were locked up in individual kennels. The other animals were kept indoors in wire cages.

Normally, 10 to 20 plerocercoids were fed to each host, but in cases in which the effect of crowding was studied, as many as 200 were fed to pups and kittens, and in one case, as few as two were fed a dog.

The gulls, dogs and cats voluntarily ingested plerocercoids enclosed in a piece of fish, but in some instances it was necessary to force-feed them by opening their mouths, placing the infecting material into the pharynx or on the posterior portion of the tongue, and holding the jaws together until the material was swallowed. The wolves were force-fed in this manner. Rats were lightly anaesthetized with ether, then plerocercoids enclosed in gelatin capsules were placed into the pharynx at the base of the tongue. The material was readily

swallowed when the animals regained consciousness. Only unencysted plerocercoids, or those removed from their cysts, were used.

Stool examinations, done by standard concentration techniques, were performed on each animal from the fifth day after feeding. These were continued daily, until the animal was sacrificed or until eggs were observed. In the latter case, twice weekly examinations were thereafter conducted until eggs were no longer found in the feces.

The standard time for sacrificing animals was 15 days after infection, but in growth-rate studies, they were sacrificed or dewormed at 5-day intervals up to 35 days after infection; some were also sacrificed or dewormed on the first, third, and seventh days after infection. In longevity studies, some animals were sacrificed at periods up to 100 days post-infection. Gulls and rats were sacrificed after one feeding. Some dogs and cats were dewormed with Nemural then reinfected once or twice before being sacrificed. The two wolves were dewormed, then turned over to a zoo.

Euthanyl (Moore-Thompson-Clinger Ltd., Hamilton, Ontario), intravenously administered, was used to sacrifice the dogs and cats; rats were etherized; and the birds were decapitated. Only the intestines of experimental hosts were examined for adult Diphylllobothrium. Worms recovered were treated as

previously described.

Details of the procedures used in the experimental studies, are discussed, when necessary, in conjunction with the particular study.

(3) - Histological procedures

Most of the whole-mount preparations of plerocercoids or adult worms were stained in Semichon's Acetocarmine (Cable, 1951), but some were stained in Grenacher's borax carmine (Pantin, 1960), Ehrlich's haematoxylin (Baker, 1960), or carbol fuchsin (Carleton and Drury, 1957). Specimens were dehydrated in alcohols, cleared in xylene, and mounted in Canada balsam.

Seven to ten micron thick transverse and sagittal sections were cut from paraffin-embedded tissues. These were stained by the Mallory-Heidenhain Rapid One-Step technique (Cason, 1950), slightly modified. The staining period was extended from 5 minutes to 15 minutes. After a brief washing in tap-water, the slides were taken directly to absolute alcohol (or destained in 90% alcohol if sections were heavily overstained), then into two changes of a 1:1 solution of absolute alcohol-xylene, before clearing in xylene and mounting in DPX (British Drug Houses, London). Good results were obtained with larval and adult tissues. The Heidenhain Azan method (Pantin, 1960), which gave good results with plerocercoids but not with adult worms, and Masson's Trichrome (Pantin, 1960), and carbol fuchsin (Carleton and Drury, 1957), both of which gave satisfactory

results with both types of tissues, were also used.

TAXONOMY

During the period of study, seven distinct morphological types of plerocercoids were recognized. Due to the inadequacies of the literature, most of those could not be identified from larval characteristics alone, so adult worms were obtained through feeding distinct plerocercoid types to individual hosts. Five species were then identified by a combination of larval and adult characteristics, namely, Diphyllbothrium dendriticum (Nitzsch, 1824), Diphyllbothrium ditremum (Creplin, 1825), Diphyllbothrium osmeri (von Linstow, 1878), Diphyllbothrium cordiceps (Leidy, 1872) and Diphyllbothrium latum (L). The other two plerocercoid types could not be identified to species.

Since variations due to the age and intensity of infection, and to the species of host, are known to occur in many of the adult features commonly used in the taxonomy of this group, experiments were designed to provide information on age-dependent, host-induced, and density-determined morphological variations. But possible "geographical variations" could not be ascertained since only local populations were dealt with.

The incompleteness of the published descriptions of many species of Diphyllbothrium, and the wide discrepancies between descriptions of the same species given by different authors, make the difficult problem of correctly identifying the species of the genus even more complex. Many species are so inadequately

characterized, or were established on such inadequate material as to render comparison with them virtually impossible or meaningless. No attempt is therefore made to compare the species of this study with such species. Likewise, the species reported from marine mammals, as well as some Russian species of dubious validity, are not discussed. Comparison will be limited to well-characterized or fully described species which in some way resemble the author's material.

Descriptions of the adult worms are based on mature 15-day old specimens (30-day old specimens for D. latum), raised in dogs. The size range of the worms, the size of the scolex, and the dimensions of proglottids from different parts of the strobila are all taken from specimens raised in dogs. The characteristics of the testes and vitellaria are based on no less than 100 observations from whole-mount preparations of each of at least five different specimens. At least 5 cirrus pouches from each of 5 specimens were measured in sagittal section. In order to maintain a reasonable basis of comparison, measurements of the testes, vitellaria and cirrus pouch were taken only from proglottids in the middle portion of the strobila. Observations on histological characteristics were made on a large number of sections taken from at least 5-10 specimens (three specimens of D. latum).

The external measurements of the plerocercoids of

D. dendriticum and D. ditremum are based on over 100 specimens of each; those of D. osmeri and D. cordiceps on 40-50 specimens; those of D. latum on 20 specimens; and those of Diphyllbothrium sp.I and Diphyllbothrium sp.II on about 15-20 specimens each. Histological features were observed from numerous sections.

Data on the three coracidia described are based on numerous newly-hatched specimens killed and fixed in 70% alcohol or 1% osmic acid; the egg size was taken from several hundred eggs obtained from the stool of experimental hosts.

Although the descriptions of the adult worms are based on specimens raised in dogs, they fit specimens raised in other hosts except where noted. It was necessary to use specimens from dogs because only from this host were sufficient specimens of the same age obtained to allow proper comparisons.

Since a few new terms, such as outer and inner cortex, cortico-muscular area, intermediate zone, and central zone have been introduced, explanatory diagrams, (Figs. 5 and 6) are included.

Though the titles of three recent papers, (Vik, 1964; Stunkard, 1965; and Meyer, 1966), suggest an evaluation of the criteria commonly used in identifying the species of this genus, no attempt has been made in these works to examine these criteria critically. It was therefore thought desirable to explore this subject, especially since reasonably adequate numbers of

specimens from five, possibly seven, species, all similarly handled, were available.

A - EVALUATION OF TAXONOMIC CRITERIA

The characteristics used in differentiating the species of this genus may be grouped into three broad categories:

(a) External morphometric features, such as size and shape of strobila, scolex, and proglottids from various parts of the strobila, and length of the neck; and

(b) Internal features, which include morphometric characteristics of the components of the reproductive and the muscular systems; and

(c) Larval characteristics, such as the external and internal features of the plerocercoid, and some coracidial characteristics.

Since external morphometric features may be subject to considerable variation due to environmental and other factors, assessment of their value from a taxonomic standpoint deserves close consideration. Many of these characteristics are now generally considered unreliable, but some reputable students of the genus still assign diagnostic value to them. For instance, the length and breadth of the strobila, and the number of proglottids comprising it, are among the characteristics of the adult worm which Kuhlöw (1953b) said "besondere Beachtung bei

einer Artbestimmung verdienen"; and "the shape of the segments throughout the strobila", is among the nine features which Markowski (1949) regards as diagnostic of the species.

The Strobila

In the present study, size of strobila was so variable that it was, per se, of no diagnostic value. Nevertheless, the relative size of worms of different species reared under comparable conditions, was generally valuable in distinguishing between certain species. Under such circumstances, both the length of the strobila and size of the proglottids of D. cordiceps were always much greater than those of D. osmeri and D. ditremum. It was therefore possible at all times to distinguish between the former and either of the other two species on the basis of size alone; but this criterion could not be utilized to differentiate D. ditremum from D. osmeri. However, even in experimentally-raised specimens, so much intra-specific variation occurred that no practical taxonomic use can be made of size.

On the contrary, the mature strobila of each species studied, exhibited a characteristic gestalt or overall general appearance. Thus a large, thick, rugose, spindleform and markedly serrated strobila of proglottids which change in shape posteriad from trapezoidal through squarish to oblong, and whose lateral margins are often concave, is a distinguishing trait of D. dendriticum (Fig. 8). Like that of D. dendriticum,

the strobila of D. ditremum is thick, rugose, and serrated, but the lateral margins of its proglottids are never concave but slightly convex in the more posterior units. The small, flat, attenuated strobila of D. osmeri could not be mistaken for that of any other species studied. Immature and some early mature proglottids are trapezoidal, but the others are rectangular with rectilinear or slightly convex lateral margins (Fig. 14). Similarly, a large, slightly serrated strobila with rectangular proglottids which are much wider than long, is characteristics of D. cordiceps; and a relatively broad, ribbon-like strobila comprised of rectangular proglottids more or less uniform in width throughout its length, is significant of D. latum.

These characteristics developed with maturity; immature strobilae all looked alike, but by the time full maturity was reached, the characteristic form of each species was acquired and thereafter retained. There was no evidence in this study of the regular development of primary and secondary strobilae, reported by other workers for two of these species. Wardle and McColl (1937) described two types of strobilae for D. latum from dogs: a primary strobila of elongate proglottids, and a secondary one of proglottids which are broader than long. Replacement of the primary strobila by the secondary normally commenced about three weeks after infection, but in cases of "superinfested" hosts, was delayed for several months. Kuhlow

(1933c) observed a similar sequence in D. dendriticum, but his "type A" strobila, (the primary strobila), was short-lived, being usually completely replaced by the "Type B" in 14-18 days. Talysin (1934) reported two strobilar types for D. minus. But Rausch (1954) found no evidence of primary strobilae in 10-day old specimens of "superinfested" bears, though he observed a "primary-type" segmentation persisting for up to 100 days in dogs and foxes which were fed "plerocercoids from Salmo gairdneri". He therefore concluded that "it does not appear...that the primary type of segmentation takes place during the growth of the cestode of bears on Kodiak Island [namely, D. ursi] [since] the youngest specimens, from naturally infected bears, had all segments of greater width than length, when total length of strobila ranged from 18-30 mm."

In the present study, "primary strobilae" were observed only twice: a typical primary strobila of D. latum, as defined by Wardle and McColl (1937), was observed in a specimen reared in a cat for 15 days; and a specimen of D. dendriticum, spontaneously eliminated by its gull host on the 8th day after infection, exhibited what might be called a "primary-type" segmentation. The strobila of a second D. dendriticum, obtained when that gull was sacrificed two days later, was typical of the species. Nothing which fitted the description of a primary strobila was observed in any other case, not even in cases of

heavy infections. It therefore appears that, as in D. ursi, primary segmentation does not normally occur in these species. Such strobilae might be induced by differences in the host's nutritional or general physiological state. It is interesting to note that in the case in which this type of strobila was definitely observed, the cat host was in oestrus during most of the period of infection, and was not feeding normally. Likewise, the D. dendriticum from gull, its normal host, was eliminated much earlier than usual.

These observations strongly indicate that, at least in the species under consideration, the shape and gestalt of the strobila are of diagnostic value, since they remain quite stable, varying only within recognizable limits, irrespective of species of host or of age and intensity of infection.

The Neck

Markowski (1949) found a "neck" consistently present in specimens of D. dendriticum but absent in D. ditremum.* He concluded that this region was of "systematic value only in so far as it was present or absent, because the length of this part of the body varies considerably, even in the same species...".

On the contrary, Rausch (1954) found that the presence or absence of a neck had no diagnostic significance in D. ursi

*In his Figs. 7 and 8 of D. ditremum (p. 115), a short neck is shown.

since it varied with the age of the specimen. A well-defined neck of variable length was present in young strobilae, but it progressively decreased in length, and eventually disappeared as the worm aged.

No such age-correlated variation in the length of this region, or any similar variation which could be attributed to the species of host, or to the intensity of infection, were found in this study. Accordingly, the relative length of the unsegmented neck region was useful in differentiating D. cordiceps, D. dendriticum, and D. latum, in which it is relatively long, from D. ditremum and D. osmeri in which it is very short - so short that in some cases it appears to be absent. In D. ditremum, its length usually does not exceed that of 2-4 of the proglottids which immediately follow it, but it is often somewhat longer in D. osmeri.

The Scolex

Both Markowski (1949) and Kuhlow (1953b) attach specific significance to the size and the shape of the scolex. Rausch (1954) observed that irrespective of strobilar size, the size of the scolex of D. ursi remained relatively uniform, but that its shape depended on the state of relaxation at the time of fixing. Wardle and McColl (1937) found much the same constancy in the size of the scolex of D. latum obtained from dogs and foxes in Manitoba and from experimentally infected kittens; but

observed that as the worms aged, the shape of the organ changed from narrowly lanceolate to broadly spatulate. In the author's material, both the relative size and the shape of the fully relaxed scolex were useful in identification, but the shape was more useful since it remained relatively constant in mature specimens of all ages grown in the different species of hosts used, except in D. dendriticum, in which the scolex was smaller in specimens reared in gulls than it was in those grown in mammals. In all other cases, only slight variations were observed.

Since Lühe (1899) introduced the use of internal anatomical features, such as the number of uterine loops and the number and size of the testes, into species identification, a large number of supposedly taxonomically important features has accrued. The shape, number, dimensions, and arrangement of the components of the genital complex, the size of the egg, and various histological features have been added to the list of criteria.

Vergeer (1942) and Thomas (1946) included in their descriptions the measurements of the ootype, the seminal receptacle, and the genital openings. Markowski (1949) regarded the number of testes seen in sagittal and transverse sections, the modifications of the uterus occurring in the "hinder portion"

of the strobila, the relation of the uterine loops to the cirrus sac, the relation of the cirrus sac to the anterior border of the segment, and the shape of the ovary, as specific criteria; and also discussed the usefulness of the state of development and the arrangement of the longitudinal muscle fibres as seen in transverse sections. Kuhlow (1953b) included some of these in his ten important characteristics of the adult worm, but considered the shape of the ovary, the shape of the shell-gland, and the relationship of the testes and the vitellaria to the uterine loops and the cirrus pouch, to be among the most important.

After examining the taxonomic value of each of these multifarious properties, the author could not help agreeing with Wardle and McColl (1937) who state that "there has been a tendency in recent years for students of tapeworm taxonomy to provide meticulous micrometric measurements of such internal structures as the cirrus sac, testes, germarium, vitellaria and genital ducts, and exhaustively detailed descriptions of the cuticle, subcuticle, musculature, parenchyma and so forth. Valuable as such ponderous and scholarly descriptions must be to the student of comparative histology, it may be doubted whether they are of like value to the taxonomist...". In the present study, many of these characteristics have been found to be of no consequence whatsoever as diagnostic features;

others seemed desirable merely as descriptive attributes but not as specific criteria; and some others had limited or definite systematic value.

The Testes

The number, size and shape of the testes are of questionable validity as taxonomic criteria. Markowski (1949) included these, as well as the distribution of the testes among the "features which may be regarded as specific criteria", (p. 110), but later, on p. 119, partly contradicted himself by asserting that the number of testes was too variable to be of systematic value, and that their size depended on the degree of development. Korpaczewska (1958), considered the number, size, and distribution of the testes as essential features in the identification of D. dendriticum from Larus ridibundus and Larus canus in Poland. The author's findings agree with Rausch's (1954) conclusion that distribution, but not number and size of these structures "can be relied upon as being fairly uniform for a given species". Their distribution in relation to the uterine loops, the ovary, and the cirrus pouch, and whether or not they are confluent anterior to the cirrus pouch and/or posterior to the ovary, are species-specific characteristics; but the number per segment is so variable that it is totally useless as a systematic criterion. Even in a single worm the number in one proglottid may be more than

twice that in another; and proglottids in the posterior half of the strobila generally contain a significantly higher number than do early mature proglottids. In like manner, the number seen in sagittal and transverse sections (included among Markowski's criteria) is too variable to be meaningful. Their shape and orientation showed some measure of stability in D. dendriticum in which they are usually ovoidal with their long axes horizontally arranged.

The Vitellaria

Much the same points raised with reference to the testes hold in the case of the vitellaria: only their distribution may be relied upon as stable, their number, size and shape being rather inconsistent.

The Uterus

The number of uterine loops on either side of the midline, though reasonably stable, is of doubtful utility since there is so much overlap between species, and since a higher number always occurs in proglottids of the posterior portion of the strobila. However, the arrangement of the loops, and the shape and width-length ratio of the complex, seem to be characteristic. The uterus of D. latum is a long, narrow, asymmetrically branched unit which is twice as long as broad, while that of D. osmeri is in the form of a loose spiral whose length-width ratio is

2:1 or greater. Those of D. dendriticum and D. ditremum are rosette-shaped in the central portion of the strobila. The loops in some cases are relatively large, and are generally symmetrically and compactly arranged. Its length-width ratio varies according to the shape of the proglottid, but is usually 1:1 to about 3:2. In D. cordiceps, the complex is often very prominent as a broad, tree-like structure with a width-length ratio of 1:1 and loops which are well-separated from one another. The relationship of the anterior-most pair of uterine loops to the cirrus pouch is also quite stable except for differences observed in D. dendriticum from gulls and mammals (discussed more fully under D. dendriticum, below). This feature is therefore of systematic significance.

The Ovary

Kuhlow (1953b) attached such great significance to the shape of the ovary that he suggested it might be the most valuable characteristic in species diagnosis. The author agrees, though with certain reservations, that the shape of this organ has considerable diagnostic value. Its shape in D. ditremum and D. osmeri is relatively constant, and its structure in D. latum and D. cordiceps is peculiar to these species. In D. dendriticum, however, its shape is not constant throughout the length of the strobila. Whereas in proglottids of the anterior and middle portions of the strobila the organ is

characteristically broad, thick-lobed and somewhat butterfly-shaped with poorly developed anterior horns, in segments of the posterior portion, it is roughly H-shaped with somewhat crescentic lobes whose anterior and posterior horns are well-developed (Fig. 7).

The Cirrus Pouch

Not much mention is made in the literature of the possible diagnostic value of the cirrus pouch. One would expect that such a large and relatively muscular organ might provide some useful taxonomic characteristics. Its size, volume, surface area, shape, orientation, and extent of projection into the medulla have therefore been studied from scores of sagittal sections of each species.

In D. latum several of these factors were relatively constant, but in the other species, only the shape of the organ appeared to be sufficiently uniform to be of some taxonomic significance. The structure in D. latum is very large, always pyriform, and extends completely across the medullary parenchyma to the dorsal musculature (Fig. 21). In D. ditremum, it is usually roughly spherical to ovate, and projects to varying extents into the medulla, but never completely across it.

The shape and orientation of the cirrus pouch in D. dendriticum and D. cordiceps are determined to a great extent

by the degree of development of the large sacculate anterior pair of uterine loops, which is often closely applied to it, and in some cases, tends to surround it. Such loops, bulging with eggs, push the unanchored end of the pouch anteriorly causing it to assume a position ranging from diagonal to nearly perpendicular to the dorso-ventral axis of the proglottid. Its normal ovoid or pyriform shape in D. dendriticum often becomes distorted through pressure from these loops. In D. cordiceps, the shape is quite inconsistent and may be spherical, ovate, and pyriform in consecutive proglottids. In both D. dendriticum and D. cordiceps, their extent into the medulla varies from half-way to more than three-fourths across. The normally ovate or pyriform pouch of D. osmeri extends from about four-fifths to completely across the medulla.

In all species, the volume and the surface area of the cirrus pouch are extremely variable from proglottid to proglottid. Its large size in D. latum clearly separates this species from any of the other species studied, but there was too much variation and too much interspecific overlap for size to be relied upon as a diagnostic feature in the other species. Only limited specific value may therefore be assigned to the cirrus pouch..

The Muscle Layers

As far as the author could ascertain, only Markowski (1949) mentioned the apparent systematic value of the degree

of development and the arrangement of the longitudinal musculature of the adult worm. He did not include these as specific criteria, but pointed out the comparative development and arrangement in D. dendriticum, D. ditremum, and D. latum as seen in transverse sections.

In the present study, the development and arrangement of the longitudinal muscles were among the most stable characteristics of the adult worm, and provided a sound basis for separating D. latum, in which the fibres are grouped into distinct and separate bundles, from D. dendriticum, in which they are arranged in large, contiguous bundles forming a deep layer, and from D. ditremum and D. osmeri, in which they exist mainly as individual fibres in a shallow, poorly developed layer. In D. cordiceps, they are always in bundles, but the arrangement is transitional between that of D. latum and that of D. dendriticum - in one part of the section the bundles are isolated from one another, in another part they are contiguous (Fig. 18). In sagittal sections of D. latum, D. dendriticum, and D. cordiceps, these fibres are seen as a relatively wide and well-developed layer: in sections of D. ditremum and D. osmeri, they form a much narrower layer. (See Figs. 9, 12, 15, 18, 21).

The Egg

Several workers have discussed the taxonomic value of

the egg in this genus: Magath (1929) found that in D. latum from Minnesota and Manitoba, young worms produced eggs which ranged in size from $52-65\mu$ by $36-42\mu$ (mean 57 by 39μ), while eggs from old worms measured $55-76$ by $46-52\mu$ (mean 64 by 47μ). He subsequently concluded (Magath, 1933) that the eggs of the species of this genus are too similar to be of diagnostic value. Faust (1952) reached the conclusion that the size range of D. latum eggs reported by various European workers "leave [sic] no doubt as to the uselessness of citing egg measurements" for this species. Rausch (1954), after suggesting that variations in size of eggs of Diphyllbothrium spp. may be related to geographical distribution of the species, concluded that "...differences of reasonable degrees in size and proportions of eggs may have value in distinguishing species of Diphyllbothrium".

In his study of eight species of Diphyllbothrium, Hilliard (1960) found that species "can be separated according to the environment of the last intermediate host (viz, fresh-water, brackish, or marine)", on the basis of the size and width-length ratio of the egg, the thickness and nature of the egg shell (smooth versus scrobiculate), the nature of the opercular suture (incised or smooth), and the presence or absence of an apical knob. He also concluded that "mean measurements of eggs are significant if only fertile ones are

considered and if, for the same species of cestode, the final hosts are of the same class (i.e. either a mammal or a bird)". Meyer (1966), in his comprehensive summary of specific criteria in this genus, concluded that in the absence of statistical analysis of the aforementioned factors, the egg is of little value in species identification.

In the present study, egg size was partially useful in separating species such as D. latum and D. cordiceps which have large eggs, from those such as D. ditremum and D. osmeri which have small eggs. Whether the host was a mammal or a bird had no effect on the size range or the mean size of the egg, no correlation between egg size and age of the egg-producing worms could be found. Though the size range seems stable, the interspecific overlaps which occur, as well as similarities in colour, in the nature of the shell, and of the opercular suture, rendered the egg of little value in species diagnosis.

Larval Characteristics

In his pioneering work, Kuhlow (1953a) emphasized the importance of external morphological features such as the nature of the cuticle, and the size and shape, of the plerocercoid, as well as of histological characteristics, such as the thickness of the cuticle, the presence or absence of cuticular bristles,

the state of development and the arrangement of the parenchymal and subcuticular musculature, and the distribution of the frontal glands.

The value of these characteristics were assessed in the present study, and those which were found to be the most valuable because of their stability were

1. The shape of the plerocercoid;
2. The distribution of its frontal glands;
3. The nature of its cuticle; and
4. The state of development and arrangement of its longitudinal parenchymal musculature.

These characteristics were quite constant. The shape of the body and of the scolex, as well as the distribution of the frontal glands, was quite uniform among members of the same species irrespective of method of preservation and preparation.

The nature of the cuticular surface - wrinkled versus smooth, and bristle-covered versus bare - was very constant, but as Wikgren (1964) observed in D. dendriticum and D. osmeri, the cuticle varied in thickness, with the ranges in D. dendriticum, D. ditremum, D. cordiceps, and D. latum overlapping.

The arrangement of the longitudinal parenchymal muscle fibres into well-defined bundles is a stable feature of D. osmeri and D. cordiceps. These fibres tend to be separated into loose bundles by single dorso-ventral fibres in D. dendriticum and D. ditremum. In D. latum, they always occur as single units.

The exact thickness of each layer seen in transverse section is unreliable, since this depends on the size of the plerocercoid, but the thickness of each, relative to the others, seems to be reasonably uniform. Similarly, the length of the cuticular bristles varies widely, with overlapping ranges in some species. Wikgren (1964), however, found it convenient to divide the adequately described plerocercoids into three groups on the basis of the length of their cuticular bristles, namely, those without bristles, those with bristles 3-14 μ long, and those with bristles more than 14 μ long. In his first category should now be included D. cordiceps, and in the third, D. ditremum.

The taxonomic significance of encystment and of location of plerocercoids in their hosts, have been discussed by several authors: Wardle (1935) states that "it can be taken as certain, ...that any plerocercoid that is enclosed in a cyst is not Diphyllbothrium latum". Eguchi (1933) and Wikgren (1964) have reported encysted larvae of this species. In the present investigation, as well as in many others, (Duguid and Sheppard, 1944; Hickey and Harris, 1947; Vik, 1957; Fraser, 1960a; and Wikgren, 1964, inter alia), plerocercoids of the same species were often found both encysted and unencysted in the same fish specimen, and even in or on the same organ. This conclusively demonstrates the

uselessness of this characteristic.

Kuhlow (1953a), who found D. vogeli plerocercoids only in the liver of sticklebacks (Gasterosteus sp.), Vik (1957), who regards the habitat differences between D. norvegicum and D. cordiceps as one of their most important differences, and Dubinina (1962), who postulates plerocercoid location as an evolutionarily stabilized host-parasite relation, seem to assign considerable taxonomic importance to the location of the plerocercoid in its host. On the basis of evidence presented in Table 18 on the location of the same species of parasite in the salmonids of Kootenay Lake, the author must conclude that location in host has no taxonomic significance.

Fraser (1960b), and Hilliard (1960) hold somewhat opposite views on the taxonomic significance of coracidial hooks. On the basis of the limited amount of work done on coracidia in the present study, the author agrees with Hilliard that hook size and form are too variable to be of diagnostic value. Size of the organism, which was the only other characteristic examined for possible taxonomic clues, was also too variable to be of any value.

B - SPECIES DESCRIPTION

Diphyllbothrium dendriticum (Nitzsch, 1824) Lühe 1910.

Synonymy: Bothriocephalus dendriticus Nitzsch, 1824.

Dibothrium dendriticum of Diesing, 1850.

Dibothriocephalus dendriticus of Luhe, 1899.

Diphyllbothrium canadense Cooper, 1924.

Diphyllbothrium sp. of Markowski, 1933.

Diphyllbothrium norvegicum Vik, 1957.

(i) The adult stage - Figs. 8 and 9

Strobila thick, rugose, serrated, and somewhat spindle-shaped, 32 to 215 cm. long by 8 to 9 mm. in greatest width. Proglottids of anterior $3/5$ of strobila wider than long and trapezoidal in shape; early mature proglottids with width-length ratio of 4:1, more mature ones with ratio of 3:1, proglottids in penultimate portion squarish and those in terminal fifth oblong with width-length ratio of 1:2 or 1:3; lateral margins of proglottids of posterior half often concave with prominent anterior and posterior bulges.

In fully mature segments, uterine complex rosette-like, about as long as wide; consists of 5-8 pairs of loops (up to 9-10 in posterior segments); distal 4-5 pairs with ripe amber-coloured eggs, others with unripe eggs; anterior-most pair

sacculate, usually extends up to anterior border of cirrus pouch but often surrounds same.

Ovary somewhat butterfly-shaped and situated in posterior portion of segment; consists of two lateral lobes joined closer to anterior ends by narrow isthmus; reticulate in appearance; anterior horns poorly developed, posterior horns approaching each other, sometimes meeting at midline; anterior horns extend anteriorly to first uterine loop containing ripe eggs. Width-length ratio of organ 2:1 in proglottids of mid-portion of strobila, 4:3 in posterior proglottids. Mehlis' gland crescent shaped, located posterior to isthmus between posterior horns of ovary.

Vitellaria numerous, of variable shape and size: irregularly spherical, ovoid, reniform or pyriform, and measuring from 36 by 36 μ to 72 by 51 μ , but usually 44-51 μ by 44 μ ; distributed in dorsal and ventral cortical areas in lateral fields co-terminal with tips of uterine loops, seldom overlapping them, and continuous from proglottid to proglottid; usually extend across midline anterior to cirrus pouch, but often do not, and seldom cross midline posterior to ovary.

Vagina slightly convoluted, runs anteriad from isthmus of ovary, ventral to uterus, up to vicinity of genital opening, there turning dorsad and extending to area of seminal vesicle before turning around and passing along posterior border of cirrus pouch to enter genital sinus posterior to male opening.

Testes abundant, of variable shape and size: mainly ovoid with long axes horizontal, but also spherical, reniform and pyriform, and measuring from $102 \times 87 \mu$ to $188 \times 102 \mu$, usually $116-145 \mu$ by $87-102 \mu$; arranged in single medullary layer in lateral fields, occasionally overlapping the ovary, generally contiguous with tips of uterine loops but rarely overlapping them; fields may or may not be confluent anterior to cirrus pouch but never confluent posterior to ovary. Both testes and vitellaria so distributed as to leave free area anterior to cirrus pouch and often around uterine complex.

Cirrus pouch in anterior quarter of segment, closer to its anterior boundary in early mature proglottids than in older ones; in sagittal sections, irregularly ovoid in shape, weakly muscular, extends approximately half-way to two-thirds across medulla; in fully gravid segments antero-dorsal margin directed forward; measures from $290-420 \mu$ by $160-290 \mu$. Ejaculatory duct enters postero-dorsal wall of sac. External seminal vesicle ovoid in sagittal section, muscular, and at its postero-dorsal border receives convoluted vas deferens which runs forward from vicinity of ovarian isthmus dorsal to uterus.

Genital opening in midline in anterior portion of proglottid; uterine pore opens short distance posterior to same and usually to one side of midline.

Longitudinal parenchymal musculature well-developed:

fibres in deep layer arranged in bundles, transverse musculature also well-developed - both layers readily seen in transverse and sagittal sections. From transverse sections, ratio of outer cortex: inner cortex: muscle layers: medulla = 1:2:2:3; cortico-medullary ratio = 1:1.

Neck region usually few millimetres long and distinctly unsegmented. Scolex mainly lanceolate or ovoid and measures from 2.0 to 2.5 mm. long by 0.5 to 0.8 mm. wide (mode 2.0 by 0.7 mm.); but much smaller in specimens reared in gulls, in which case it measures 1.5 - 1.8 mm. in length by 0.5 to 0.7 mm. in width, with a modal size of 1.7 by 0.6 mm. Bothria extend length of scolex.

Hosts:

Natural - ring-billed gull, California gull, herring gull, common merganser.

Experimental - dog (93.6% of 33 feedings), cat (73.7% of 19 feedings), rat (56.2% of 17 feedings), wolf (one feeding), young ring-billed gull (54.2% of 24 feedings). None of the six human volunteers became infected.

(ii) The plerocercoid stage - Fig. 10.

External features

Coarse, glistening white or creamish worm measuring from about 1.0 cm. to 30 cm. in length, and from 0.75 mm. to 3.0 mm. broad (mode: 3.5 cm. to 5.0 cm. by 1.0 - 1.5 cm. when in viscera, and 7.0 - 8.0 cm. when in muscle). Body thick and deeply wrinkled, giving appearance of incipient segmentation; width of body

approximately uniform along length but slightly wider toward posterior. Posterior extremity usually rounded and bearing marked invagination. Scolex laterally compressed; mainly ovate or ovate-acuminate in lateral views; always evaginated or only partially retracted; 0.75 to 1.5 mm. long by 0.5 to 1.0 deep - mode: 1.5 x 0.75. Bothria extend full length of scolex thus giving scolex gaping appearance at summit.

Internal features

Transverse section through body and scolex ellipsoidal. Cuticle about 19 μ thick; cuticular bristles very short, 3-5 μ , and difficult to discern, or lacking. Frontal glands weakly developed, distributed in pockets throughout scolex and extend into anterior part of body - extent of distribution better seen in sagittal section (Fig.10d). Subcuticular muscle fibres in single narrow layer about 7 μ wide. Layer of subcuticular cells much wider, from 50 to 60 μ in width. Parenchymal musculature well-developed: longitudinal fibres form layer about 130-160 μ thick, fibres only loosely separated into bundles by single dorso-ventral fibres; transverse fibres in layer 29-44 μ wide. Intermediate zone, between parenchymal muscle and subcuticular cell layer, about 1/2 as thick as longitudinal parenchymal muscle layer, 65 to 87 μ wide. Central zone about same width as longitudinal parenchymal muscle layer; contains pair of lateral nerve cords lateral to main pair of excretory ducts. Numerous peripheral excretory ducts penetrate

section.

Host: rainbow trout; and occasionally dolly varden char, and kokanee.

Location in host: mostly lying free or encysted in spherical or ovoid thin-walled cysts, 4 mm. to 10 mm. in greatest dimension, on serosa of stomach, in perigastral fat, on or among digestive caeca, in or on gonads, and on retroperitoneal surface of liver; sometimes encysted in liver, spleen and swim-bladder; occasionally encapsulated in musculature, especially in hypaxial muscle adjacent to stomach.

(iii) The coracidium - Fig. 25

Hatches in 6-8 days when egg incubated at 20°C.; actively swims about in tumbling rotary manner; remains active at room temperature for 25 to 36 hours, gradually decreasing in activity and dying after 50 to 60 hours. Ellipsoidal when hatched but shortly thereafter assumes spherical shape; 36 to 72 μ in diameter on hatching, usually about 45 to 54 μ , but rapidly increases in size, attaining twice original diameter within hours.

Ciliated embryophore up to 11 μ thick surrounds spherical oncosphere. Cilia up to 44 μ long at anterior pole, up to 20 μ long at opposite pole. Oncosphere carries three pairs of hooklets at one end, 12 to 13.5 μ long, the middle pair being somewhat shorter; tip of blade of middle pair moderately curved; ratio of blade length to length of handle 1:3 to 3:8.

(iv) The egg - Fig. 26

Amber-coloured when ripe; usually ovoidal or slightly pyriform in shape, operculum at narrower end, boss sometimes present at opposite end; 55-62 μ by 40-51 μ in size. Unembryonated when discharged, ovum almost completely obscured by granular vitelline cells which surround it. At 20°C., hexacanth larva becomes visible by fourth day; completely developed and ready for hatching by sixth to eighth day, but if incubated in darkness, normally does not hatch until exposed to light. Remains fertile for over two years when stored at 40°C.

(v) Morphological Relationships

The adult specimens fit the descriptions given by Markowski (1949) and by Kuhlowl (1953c). The dimensions of the testes recorded in these three studies do not, however, agree completely, although they fall roughly within the same range: Markowski gives their diameter as 99-165 μ by 62-148 μ ; Kuhlowl gives it as 53-110 μ by 28-75 μ ; and in the author's specimens, they measure 87-188 μ by 72-116 μ . The plerocercoid answers the description of Kuhlowl (1953a,c) and of Wikgren (1964), and is identical with the figure of the worm referred to by Vergeer (1942) as Sparganum pseudosegmentatum.

The author's material also bears a remarkable similarity to the adult and plerocercoid stages of D. norvegicum. Vik (1957) has stressed this resemblance between the two species, and has indicated that the real difference between them could

be determined only from the results of life-cycle studies. He concluded from his experiments, that unlike the plerocercoid of D. dendriticum which requires a single fish host (Kuhlow, 1953c), that of D. norvegicum must enter a second such host in order to develop into the infective stage. The author cannot say what is the exact situation in the case of his species, but good circumstantial evidence seems to suggest a situation similar to that of D. norvegicum.

The author could not find any real difference between D. dendriticum and D. norvegicum. The plerocercoids are identical, except for a few small differences, the most important of which is the absence of cuticular bristles or the presence of very short ones (3-5 μ long) in D. dendriticum. In most specimens, the posterior ends of D. dendriticum is slightly indented but the opposite condition exists in D. norvegicum. Apart from the longitudinal parenchymal musculature which in D. dendriticum is somewhat more developed than in D. norvegicum, their histology too is identical. A feature shared by the two, but which was not reported by Kuhlow (1953a), is the extension of the frontal glands into the anterior portion of the body. This is best seen in sagittal sections (Fig. 10d), and might have been overlooked by Kuhlow.

In view of the close morphological and histological similarities between the two plerocercoids, Wikgren (1964)

suggested that the two species be synonymized. The author agrees with this view, especially since in appearance and details of anatomy the similarity between the adult worms is similarly striking, the shape of the cirrus pouch being the major difference.

In many respects the plerocercoid of D. dendriticum resembles that of D. sebago (Ward, 1910) as pictured and described by Meyer and Robinson (1963). They are similar with respect to the arrangement of the subcuticular muscle fibres, and the development of the longitudinal and circular parenchymal musculature; but they differ especially in mean size (15 mm. in D. sebago), and in the absence of frontal glands in the scolex of D. sebago. The adult stages are dissimilar, especially in strobilar size and the arrangement of the uterine loops, which, in D. dendriticum form a rosette-like complex but in D. sebago looks more like a symmetrically branched tree.

D. dendriticum cannot be confused with D. latum from which it differs in many respects, especially in the shape of the strobila and scolex, the shape of the proglottids, the structure of the uterus and of the ovary, and the distribution of the vitelline glands and of the testes. Their plerocercoids, too, differ in appearance, morphology, and histology: whereas in D. dendriticum the scolex is always evaginated, in D. latum it is nearly always invaginated; both plerocercoids have

wrinkled bodies, but this is more pronounced in D. latum; the cuticle in the latter is always without bristles; and in that species, the frontal glands extend much further into the body than they do in D. dendriticum.

D. dendriticum can be readily separated from D. ursi Rausch, 1954 by the absence of the neck in mature strobilae of the latter, by the shape of the ovary, the large size of the cirrus pouch of D. ursi, and in having plerocercoids which differ entirely in external features and in histology. The plerocercoid of D. dendriticum is larger and more robust than that of D. ursi. In the latter species, the frontal glands are well-developed, arranged in connected dorsal and ventral masses, and are restricted to the scolex; the cuticle is thin (4-5 microns) and smooth or only slightly wrinkled; and the muscles of the parenchyma are arranged in large bundles. The opposite conditions exist in D. dendriticum.

From D. alascense Rausch and Williamson, 1958, D. dendriticum differs noticeably in the shape of the scolex (cordate in D. alascense); in the distribution of the vitellaria, which in D. alascense are restricted to two separated lateral fields slightly overlapping the uterine loops; in the position of the anterior-most pair of uterine loops relative to the cirrus pouch (never extends anteriorly beyond middle of cirrus pouch in D. alascense); in the shape of the ovary, which is H-shaped with well-developed anterior horns in D. alascense; and in the presence of

"glandular cells" in the scolex of the adult of the latter. The plerocercoid of this species is unknown.

Rausch (1956) states "D. dalliae n. sp. resembles D. dendriticum in both strobilar and plerocercoid stages"; and went on to say that "the plerocercoid of D. dalliae n. sp. differs from that of D. dendriticum in the presence of cuticular bristles on the scolex and in histological details". From comparisons made with material obtained on loan from Dr. Rausch, the author does not think that the two species resemble at all. One of the two plerocercoids in the whole-mount preparation studied was small, measuring 9 mm. by 0.75 mm, and bears no resemblance to D. dendriticum plerocercoids. The other was large, measuring 25 mm. by 2 mm., and resembles D. dendriticum only in size, but differs from it in general appearance, shape of the body, form of the scolex, and in the nature of the cuticle (smooth in the D. dalliae specimens: wrinkled and thicker in D. dendriticum). The proglottids were different from those of D. dendriticum in shape: they were slightly rectangular or quadrate with rectilinear or slightly convex lateral margins. The uterine complex had a greater length-width ratio (2:1) than is the case in D. dendriticum, and as Rausch pointed out, the vitellaria closely surround the uterus and the cirrus pouch, thus leaving no free space around the latter as seen in D. dendriticum.

Several other North American species were reduced to synonyms of D. dendriticum by Markowski (1949). Among these are D. canadense Cooper, 1924, D. laruei Vergeer, 1942, and D. oblongatum Thomas, 1946. Cooper's (1921) figure of the scolex of the plerocercoid of D. canadense resembles the scolex of some specimens of D. dendriticum studied. Unfortunately, this scant illustration and his failure to include a description of the plerocercoid make comparison impossible. From the description and figure of a mature segment, it is clear that D. dendriticum and D. canadense are similar with respect to certain details of anatomy such as the size, shape, and orientation of the testes, the shape of the uterus, and the extension of the anterior-most pair of uterine loops relative to the cirrus pouch. But the distribution of the vitelline follicles (greatly overlapping the uterus in D. canadense), and the shape of the ovary and the scolex are dissimilar. Though in the figure, the vitellaria, as well as the testes, are shown in close association with the cirrus pouch, in the text, Cooper states that the vitelline follicles are so distributed as to leave "free more or less circular areas above and below the uterus...". Because of these similarities, the author shares Markowski's opinion on the status of this "species".

Vergeer (1942) reared adult D. laruei in a dog from plerocercoids found in lake herrings and chubs, Leucichthys sp.

The scolex and neck region of this species somewhat resemble those of D. dendriticum, but the former differs from the latter in having a much smaller strobila, in the form of the uterus (spiral in D. laruei), the position of the anterior-most pair of uterine coils relative to the cirrus pouch (does not extend more than 1/3 along cirrus pouch in D. laruei), and in the shape and orientation of the testes. Their plerocercoids too are dissimilar. That of D. laruei is much smaller (average length 8.15 mm., range 2-26 mm.), and has a smooth body which is "elongate, cylindric or only slightly flattened, tapering from the scolex to the posterior end". A description of its histological characteristics was not included in Vergeer's paper. On the basis of these differences, the author cannot agree with Markowski that D. laruei and D. dendriticum constitute a single species.

D. oblongatum is a small cream-coloured worm found in natural infections of young gulls in northern Lake Michigan, and raised experimentally by Thomas (1946) in fledgling gulls from cysts on the stomach of lake herring. The strobila of D. dendriticum is much more robust than that of this worm. The two also differ noticeably with respect to the shape of the scolex (digitate in D. oblongatum), and the form of the uterus (as a loose spiral in D. oblongatum), and in other details of anatomy. But the two species especially resemble

each other in having tandem duplication of the genital organs in proglottids of the posterior portion of the strobila. The plerocercoid has not been described. From the available data, it appears, that at least for the time being, this species should be considered different from D. dendriticum.

Fahmy (1954) described a tapeworm recovered from an otter (Lutra lutra) in the suburbs of Edinburgh, under the name of Dibothriocephalus medius. As Sandars (1957) pointed out, this description was so incomplete that a redescription was necessary before the validity of the species could be established. This redescription was provided by Fraser (1960c) under the name of Diphyllbothrium medium (= Dibothriocephalus medius). Although she also studied "material from four natural infections...", her description was based primarily on specimens raised in rats from plerocercoids obtained from trout, but comparisons with slides of Fahmy's original material left her convinced about the identity of her specimens.

It is not possible on the basis of Fahmy's description to make proper comparisons with his species, but it may be pointed out that his figure of a mature proglottid resembles that of D. dendriticum in shape, in the general appearance of the uterus, and in the distribution of what appear to be the vitellaria and testes.

The shape of the testes in Fraser's material is unlike that of D. dendriticum (spherical in D. medium: mainly ovoid

in D. dendriticum), but their distribution is similar. Likewise, in the former, the shape of the uterus and of the ovary is not unlike the shape of these organs in some quadrate segments of D. dendriticum. The two species are also similar with respect to the ellipsoidal shape of the scolex, and the long, well-defined neck region which, however, is absent in some of Fraser's material.

Fraser did not include a description of the histological features of the plerocercoid of D. medium or a figure of the worm, in any of her papers. She merely gave its size as ranging from less than 1 mm. to 200 mm. (fixed), depending on its location in the host and on the age of the latter (Fraser, 1960c). It is therefore not possible to compare this plerocercoid with that of D. dendriticum. She also did not state whether both small and large plerocercoids were used in her experiments, and if so, whether the respective adults were similar. It is possible that at least two different species of plerocercoids were involved. If this is the case, and if both types of plerocercoids have been used, this might account for the absence of a neck in some of her specimens, the presence of a well-defined neck versus its "absence" (actually, versus the presence of a very short neck) being a reliable distinguishing feature of some species. For these two reasons, namely, this apparent weakness in this aspect of the feeding experiments

and the omission of a description of the plerocercoid, and due to the similarities between D. medium and D. dendriticum, the author believes that for the present D. medium should be regarded as a species inquirenda.

Differences between D. dendriticum and two other species recovered in this study, D. ditremum and D. cordiceps, will be discussed under the descriptions of the two latter species.

Diphyllbothrium ditremum (Creplin, 1825) Lühe 1910

Synonymy: Bothriocephalus ditremus Creplin, 1825.

Dibothrium ditremum of Diesing, 1850.

Dibothriocephalus ditremus of Lühe, 1899.

Diphyllbothrium ditremum of Lühe, 1910.

(i) The adult stage - Figs. 11 and 12

Strobila relatively thick and spindle-shaped with serrated lateral margins, 23 to 66 cm. long and 4 to 6 cm. in greatest width; somewhat smaller in gulls.

Proglottids of anterior half wider than long and trapezoidal in shape; early mature ones with width-length ratio of 3:1, and fully mature ones of 8:3 ratio; proglottids in third quarter squarish; those in the terminal quarter, square to oblong with width-length ratio of 1:1 to 1:3.

Uterine complex in stained whole-mount preparations, rosette-like and somewhat goblet-shaped in general contour; occupies central portion of segment; consists of anterior portion of 4-5 loops on each side of mid-line, containing ripe amber-colour eggs, and posterior portion of 3-4 loops with lighter coloured or semi-transparent, unripe eggs; anteriormost pair of loops sometimes sacculate and extends along lateral margins of cirrus pouch, but seldom reaches anterior border of same. Width-length ratio of complex 3:5.

Ovary in posterior half of proglottid, extends across

central 1/3 of same; somewhat dumb-bell shaped with width-length ratio of 5:3; consists of two lateral wing-shaped lobes joined about their mid-points by a narrow isthmus; both anterior and posterior horns well-developed, with anterior pair extending anteriorly to middle pair of uterine loops, and posterior ones approaching each other without meeting.

Mehlis gland crescent-shaped, situated posterior to ovarian isthmus between posterior horns of ovary.

Vitellaria numerous, of variable shape but mainly spherical or pyriform, tightly packed in dorsal and ventral cortical layers which overlap uterine loops and ovary, extend across mid-line anterior to cirrus pouch, often overlapping it, and are continuous from proglottid to proglottid; measure from 51 x 51 μ to 102 x 65 μ with a usual size of 58 x 44 μ to 72 x 58 μ .

Vagina a relatively straight thin-walled tube passing anteriorad from level of ovarian isthmus ventral to uterus; at level of genital pore it turns dorsad continuing along posterior border of cirrus pouch for some distance before turning back and entering posterior border of genital atrium.

Testes of variable size, ranging from 72 x 72 μ to 188 x 160 μ , but mainly 102 x 87 μ - 130 x 102 μ ; shape variable: spherical through reniform and pyriform to oval; distributed in single medullary layer; contiguous with uterine loops and

cirrus pouch; cross mid-line anterior to cirrus pouch but never posterior to ovary which they overlap; continuous with those of adjacent proglottids.

Cirrus pouch situated in anterior quarter of proglottid; in early mature proglottids, anterior border coincides with that of proglottid or may even protrude into adjacent proglottid; distance from anterior margin of proglottid increases posteriad along strobila; spherical in sagittal sections and extends $\frac{4}{5}$ across medullary parenchyma; and measures from 232 to 362μ by 188 to 290μ . External seminal vesicle ovoidal in sagittal section, with thick muscular wall; ejaculatory duct leaves ventral surface of vesicle and runs to posterodorsal wall of cirrus pouch. Vas deferens coiled, originates in vicinity of ovarian isthmus, passes forward dorsal to uterus to enter posterior wall of seminal vesicle.

Genital pore opens ventrally in mid-line in anterior portion of proglottids; uterine pore located few microns posterior to same.

Longitudinal parenchymal musculature moderately developed, arranged as individual fibres which show only slight tendency toward loose bundle formation; circular muscle layer of few fibres only. From transverse sections, ratio of outer cortex: inner cortex: muscle layers: medulla = 1:2:1:3; cortico-medullary ratio = 1:1. Neck region very short, usually not more than 50μ long. Scolex cordate, ovate-cordate or cordate-

lanceolate in shape, 1.6 to 2.3 mm. by 0.9 to 1.3 mm. (mode = 2.2 by 1.2 mm.); bothria extend along entire length.

Hosts:

Natural - ring-billed gulls, California gull, herring gull, common merganser.

Experimental - dog (82.4% of 34 feedings), cat (70% of 20 feedings), rat (37.5% of 16 feedings), wolf (one feeding), young ring-billed gull (60% of 20 feedings). None of the two human volunteers became infected.

(ii) The plerocercoid stage - Fig. 13

External features

Creamish or milky-white worm measuring from less than 2 mm. to about 14 mm. in length by up to 1.2 mm. in breadth - mode 5.6 mm. long by 1 mm. broad. Many variations, but basic pattern recognizable. Body relatively thick, dorso-ventrally flattened, moderately wrinkled, bears prominent "shoulder" which distinctly delimits scolex, and tapers posteriorly to sharp or blunt point which may or may not carry a posterior invagination.

Scolex laterally compressed; acuminate in dorso-ventral view, ovate in lateral aspect. Usually fully extended, measuring 0.7 - 1.0 mm. by 0.5 mm. (mode 1.0 x 0.5 mm.), but may be partially retracted, measuring 0.5 mm. or less in length. Bothria extend length of scolex.

Internal features

Cross-section through body ovidal, that through scolex oval to circular depending on level at which section taken; cuticle 11 to 14.5 μ thick in anterior portion of body, thinner

to posterior part of body; covered with cuticular bristles 14.5 μ to 29 μ long (mode = 14.5 to 19 μ), shorter on scolex; frontal glands very well developed, distributed throughout scolex and sometimes extend into anterior portion of body; subcuticular musculature in 2-4 layers, about 19 to 36 μ thick (mode 19-29 μ); layer of subcuticular cells about same thickness as subcuticular muscle layer; longitudinal parenchymal musculature well-developed, forms layer many times thicker than previously-mentioned layers (actual thickness relative to plerocercoid size), fibres separated into loose bundles by single dorso-ventral fibres; transverse muscle layer of several fibres and about 1/3 of thickness of longitudinal layer, intermediate zone and central zone approximately same width as longitudinal muscle layer; main pair of excretory ducts in central zone and median to lateral nerve cords; many dorso-ventral muscle fibres traverse sections; many calcareous corpuscles in cortical and medullary zones.

Hosts: kokanee, rainbow trout, dolly varden char.

Location in host: encysted in small, thin-walled cysts on serosa of stomach, occasionally in musculature of stomach (smaller specimens), in perigastral fat, and on caeca. Often unencysted in same locations. Cysts spherical, measure 1 to 3 mm. in diameter (mode 2-2.5 mm.), and contain only one plerocercoid each.

(iii) The coracidium - Fig. 25

Hatches in 6-9 days, usually on 7th day when egg incubated at 20°C.; spiro-rotary movement directional and accomplished by means of ciliary action; at room temperature marked decrease in activity after 25 to 36 hours, death in 50 to 60 hours. Spherical in shape, 36-54 μ in diameter, rapidly increasing in size within hours to about twice original size. Ciliated embryophore up to about 9 or 10 μ thick surrounds spherical oncosphere. Cilia at anterior pole up to 40 μ long, up to 15 μ at opposite pole. Three pairs of hooklets with prominent guard at posterior end of oncosphere, 11.5 to 14 μ long; middle pair longer than lateral ones and has markedly curved blade; ratio of blade length to length of handle 3:8 to 1:3.

(iv) The egg - Fig. 26

Amber-coloured, usually ovoidal in shape, but sometimes spherical; egg-shell smooth, operculum at one end, boss usually present at opposite end; measures 55-58 by 41-43 μ . Unembryonated when released, ovum not visible, being completely surrounded by granular vitelline cells. At 20°C., hexacanth larva becomes visible by fourth day, fully developed and ready to be hatched by sixth or seventh day, but would not hatch if incubated in darkness, until exposed to light. Remains fertile over two years when stored at 4°C.

(v) Morphological relationships

The literature is sadly lacking in adequate descriptions of D. ditremum. The species was established in 1825 by Creplin under the name of Bothriocephalus ditremus, for a form from fish-eating birds in northern Europe. It was redescribed by Matz (1892) and by Lühe (1910). Hickey and Harris (1947), on the basis of Baylis (1945), identified some specimens obtained from cormorants, shags, and heron as D. ditremum, but they did not discuss the taxonomy of the parasites. The descriptions contained in all of these papers are so scanty that they are of little taxonomic value.

Markowski's (1949) description of the adult worm is the only one which is sufficiently complete for purposes of comparison. Identification of the specimens of D. ditremum in the present study is based upon this work. The specimens agree in every important respect with Markowski's description. The measurements of some characters are not identical but fall approximately within the same range.

After examining a quantity of material obtained from birds, and recorded under 10 different species names, Markowski (1949) concluded that only two valid species of Diphyllbothrium seem to occur in birds, D. dendriticum and D. ditremum. He showed that D. ditremum could be separated from D. dendriticum on the basis of several characteristics, the most important of which

appear to be

1. the shape of the scolex;
2. the neck region,
3. the state of development and arrangement of the longitudinal parenchymal musculature of the adult worm as seen in transverse section;
4. the arrangement of the testes and vitellaria;
5. the position of the anterior-most pair of uterine loops in relation to the cirrus pouch;
6. the position of the cirrus pouch relative to the anterior border of the proglottid; and
7. specificity for certain species of birds.

The author's material confirm the usefulness of characteristics (1) to (4); (5) and (6) were not found to be stable enough in D. dendriticum to be useful as distinguishing features; and not enough data were available on (7) to permit a sound conclusion.

Regarding the relationship between the cirrus pouch and the anteriormost pair of uterine loops of D. dendriticum, Markowski (p. 114) states "...the first pair of uterine loops may extend to its (the cirrus pouch) middle, but is seldom surrounded or embedded in these loops, as is the case in fully gravid segments of D. ditremum". This holds true in most specimens of D. dendriticum reared in dogs and cats, and in those grown in a

wolf, but not in those obtained from gulls either experimentally or from natural infections. In such specimens, the large, sacculate anterior pair of loops almost invariably extends beyond the middle of the cirrus pouch which then appears wedged in between, or almost completely surrounded by them (Fig. 8). Kuhlow (1953c), Korpaczewska (1958), and Meyer and Robinson (1963) have described this relationship in their specimens of D. dendriticum. Contrary to Markowski's observation, these loops have seldom been observed to reach the anterior border of the cirrus pouch in D. ditremum.

In both of these species, as well as in other species studied, the relative position of the cirrus pouch was observed to vary according to the age and the shape of the proglottid. It is very close to the anterior margin of short, broad early mature segments, but much further removed from it in the elongate gravid ones in the posterior portion of the strobila.

The only other material with which some comparison may be made is that of Kuhlow (1953b). His description is based on a single specimen reared in a herring gull (Larus argentatus) from an unidentified plerocercoid from smelt which was supposed to be harbouring D. osmeri plerocercoids. The whole fish was fed to the bird. He suspected that another species might be involved, when Diphylllobothrium eggs were found in the bird's stool much earlier than was normal for D. osmeri.

It can be seen from Table I, in which data from the three sources, Kuhlow (1953b), Markowski (1949), and the present study are compared, that Kuhlow's specimen differs in several respects from the others. Noticeable among these are the shape of the scolex and the sizes of the testes, the vitellaria, and the cirrus pouch, Kuhlow gives the size of the testes as 35-65 μ in diameter; Markowski gives it as 180 x 165 μ - 150 x 275 μ ; and in the author's specimens the modal size is 102 x 87 μ - 130 x 102 μ (range 72 x 72 μ to 180 x 160 μ). The vitellaria measure 28 x 45 μ (Kuhlow), 72.6 - 89 by 76 - 99 μ (Markowski); and 51 x 51 μ - 102 x 65 μ with a modal size of 58 x 44 μ - 72 x 58 μ in the writer's specimens. The cirrus pouch in Kuhlow's specimen measures 160-175 x 200-230 μ ; in Markowski's it is 270 x 300 μ ; and in the author's it is 232 x 203 - 334 x 290 μ . Though exact numerical values in themselves are very unreliable parameters, it seems rather unlikely that individuals of the same species would differ so widely with respect to these characteristics.

As far as could be ascertained, a description of the plerocercoid of this species has never been published. Hickey and Harris (1947) stated that the plerocercoids of the worm which they referred to under this name are superficially similar to those described by Duguid and Sheppard (1944). Hickey and Harris's plerocercoids ranged in length from 0.2 cm.

to 34 cm. (relaxed), and were both "segmented" and "unsegmented". The latter were up to 9 cm. long and the majority of the former exceeded 4 cm. in length. No further detail was given. In the author's opinion, the plerocercoid pictured in Hickey and Harris's Figs. 5 and 6 resembles that of D. osmeri (cf. Kuhlow, 1953b, Fig. 1; Chizova et al., 1962, Fig. 1B; Vik, 1964b, Fig. 2; and Fig. 16 of the present study). Feeding experiments carried out by Vik (1964) with the plerocercoids of D. osmeri seem to reinforce this opinion. Vik stated that "the adult worms obtained corresponded in appearance to D. ditremum described by Hickey and Harris (1947) from Ireland. They did not correspond completely to the D. osmeri described by Kuhlow (1953c) [sic] nor at all to his D. ditremum...". The description contained in this study may therefore be regarded as the first complete one of this plerocercoid.

Some of the plerocercoids of D. ditremum of the present study resemble the small plerocercoid which Linton (1891a) termed the "embryo" of D. cordiceps. It is not possible, in the absence of feeding experiments or comparative histological studies, to conclude that this plerocercoid is identical with that of D. ditremum; but the striking similarity between them (cf. Linton, 1891a, Plate XXVII) strongly suggests this is the case.

The plerocercoid of D. vogeli Kuhlow, 1953 is very similar

to that of D. ditremum, differing slightly in a few details such as size, thickness of cuticle and of subcuticular longitudinal muscle layer, and length of cuticular bristles; but the adults are very dissimilar, notably with respect to the shape of the scolex (short and stubby, measuring 0.9 - 1.3 mm. long in D. vogeli); in the position of the cirrus pouch (in the second third of the proglottid in D. vogeli); in the shape of the ovary; and in the distribution of the vitelline follicles and the testes (so arranged in D. vogeli as to leave a free space anterior to the cirrus pouch, as seen in D. dendriticum).

Diphyllbothrium osmeri (von Linstow, 1878) Kuhlman, 1953.

Synonymy: Bothriocephalus osmeri von Linstow, 1878

(i) The adult stage - Figs. 14 and 15

Strobila flat, attenuated toward both ends, 20 to 42 cm. in length and 3 to 4 cm. in greatest width. Proglottids vary in shape from trapezoidal in anterior fifth of strobila, through rectangular and squarish in succeeding fifths, to oblong in terminal fifth, with width-length ratios for corresponding regions of 5:3 (early mature proglottids), 2:1, 3:2, 1:1, and 4:7 respectively; lateral margins of proglottids rectilinear or slightly convex, the strobila therefore lacks a distinctly serrated appearance.

Uterus in fully mature segments a loose spiral twice as long as wide, occupying central one fourth of proglottid, consists of 9-12 loops on each side of mid-line; proximal 3-6 pairs of loops contain only unripe eggs, and together have a racemose appearance; all loops containing ripe eggs have approximately same length; anterior-most pair of loops never extends to anterior border of cirrus pouch, seldom reaches half-way up along pouch, and usually reaches no further than one-third along same.

Ovary two crescentic lobes joined approximately at their centre by a narrow transverse band; situated in posterior fourth of segment; anterior and posterior horns well developed; anterior

horns extend to first pair of uterine loops with ripe eggs; width - length ratio between 5:3 and 4:3. Mehlis' gland semicircular, situated posterior to isthmus between posterior horns of ovary.

Vitellaria numerous, of variable shape and size, but mainly spherical or ovoid, $44 \times 44 \mu$ to $72 \times 58 \mu$, mode $58 \times 44 \mu$; arranged in dorsal and ventral fields confluent laterally, anterior to the cirrus pouch, and posterior to the ovary, co-terminal with uterine loops and always overlapping ovary; continuous from proglottid to proglottid. Vagina a slightly meandering tube running forward from level of ovarian isthmus ventral to uterus, to level of genital atrium, then turns dorsad along posterior border of cirrus pouch before turning back and entering posterior wall of genital atrium.

Testes ovoid, irregularly spherical, pyriform or reniform; arranged in single medullary layer in two lateral fields co-terminal with uterine loops, confluent anterior to cirrus pouch and occasionally posterior to ovary; 72.5 to 130μ by 72.5 to 116μ , usually 101 - 116 by 87 - 101μ , continuous into adjacent proglottids.

Cirrus pouch in anterior third of segment; relative position in all proglottids constant; ovoid in sagittal section, extends full depth of medullary parenchyma, 246 - 435μ by 174 - 290μ ; receives ejaculatory duct from spherical external seminal vesicle at its postero-dorsal angle, and opens into genital

atrium anterior to vagina. Vas deferens coiled, passes forward dorsal to uterus from origin near ovarian isthmus, to point near posterior margin of seminal vesicle, then turns ventrad for some distance along cirrus pouch before turning back and entering postero-dorsal wall of vesicle.

Genital pore in ventral midline in anterior third of segment; uterine pore opens short distance posterior to same.

Muscle layers poorly developed; in transverse sections, circular muscle as few discontinuous fibres, longitudinal layer essentially as individual fibres, better seen in sagittal section. From transverse section, ratio of outer cortex: inner cortex: muscle layers: medulla = 2:4:1:4. Cortico-medullary ratio = 1.5:1.

Neck region short, usually not more than 750 μ long. Scolex mainly spatulate, but sometimes lanceolate, 1.8 to 2.5 mm. by 725-750 μ , (mode 2.2 by 0.8 mm.). Bothria extend full length of scolex.

Hosts:

Natural - unknown.

Experimental - dog (58.8% of 16 feedings), cat (50% of 10 feedings, all immature), rat (37.5% of 16 feedings), young ring-billed gull (22.7% of 22 feedings, mainly immature).

(ii) The plerocercoid stage - Fig. 16External features

Long, slender, ivory-white worm measuring from 8 x 0.5 to 17 x 1.2 mm. Mode 9-12 mm. x 0.6 mm. Body appears cylindrical (but transverse sections somewhat rectangular), smooth, and tapers gracefully to blunt posterior point. Scolex always evaginated, is lanceolate in lateral view, and measures from 1.0 to 1.5 mm. long by 0.5 to 0.7 mm. broad - mode 1 mm. x 0.6 mm. No marked shoulder present; scolex slopes gradually, merging smoothly with body, and forming in some cases a "collar" around its anterior portion. Bothria extend full length of scolex, and are wider at the apex than at the base.

Internal features

Transverse section through body somewhat rectangular, that through scolex roundish. Cuticle 5.5 to 9 μ thick (mode 7.5 to 9 μ), and covered with cuticular bristles 11 to 14.5 μ long which cover scolex including bothrial grooves. Frontal glands well developed, confined to scolex and arranged in two lateral masses on either side of bothria. Subcuticular longitudinal muscle fibres arranged in 3-4 layers about 21-22 μ thick. Layer of subcuticular cells about same thickness as subcuticular muscle layer with large gland-like structures along medial border. Longitudinal parenchymal musculature well developed; fibres in distinct bundles. Thickness of longitudinal parenchymal muscle

layer to that of transverse muscle layer about 3:1. Width of intermediate zone to thickness of parenchymal longitudinal muscle layer 1:1. Main pair of excretory ducts in central zone and median to lateral nerve cord. Several peripheral excretory ducts present. Calcareous corpuscles in cortical and medullary regions.

Host: kokanee, rainbow trout, and dolly varden char.

Location in host: encysted in thin-walled cysts or lying free on serosa of stomach, perigastral fat, caeca, gonads, mesentery and retroperitoneal surface of liver. Cysts spherical or ovoid, and measure 1.5 to 3.0 mm. in greatest dimension. Usually there is one plerocercoid per cyst, but often 2 or 3 or even 4 worms occur per cyst.

(iii) The egg - Fig. 26

Normally ovoid but sometimes spherical, unembryonated when deposited, ovum completely obscured by vitelline cells; egg-shell smooth, operculum at one end, prominent boss may or may not be present at opposite end; measures 51-56 by 36 - 43 .

Morphological relationships

On the basis of plerocercoid and adult characteristics, D. osmeri can be readily distinguished from the other species comprising this study, and from all other adequately described

species.

Identification of D. osmeri was based to a considerable extent on the plerocercoid stage. Only a small number of mature adult worms was obtained from feeding experiments, and these differed from the only description available to the author, that of Kuhlow (1953b) with respect to the size of the scolex, the vitellaria, the testes, and the cirrus pouch, which in that author's material measure 1.15-1.75 by 690-960 μ ; 20-30 μ ; 65-75 μ ; and 180-210 μ , respectively. Although as stated previously, exact size is an unreliable criterion, such wide differences should be noted.

Conversely, the plerocercoid, and especially details of its histology, corresponded in most respects with the data provided by Kuhlow (1953a,b), and by Chizova et al. (1962). In external appearance, they are unlike that pictured by Wikgren (1964) in his Fig. 1A, but correspond satisfactorily with the data given in his Table I, and with the transverse section figured in Fig. 3B, though not with Fig. 3A*.

A prominent feature of the plerocercoid of D. osmeri is the grouping of the longitudinal parenchymal muscle fibres into well-defined bundles, a feature which is shared by the plerocercoids of D. ursi, D. dalliae and D. cordiceps, among the

*Fig. 3A is unlike Fig. 3B in the arrangement of the longitudinal parenchymal muscle fibres - singly in the former - in bundles in the latter.

fully described plerocercoids.

In many respects, the plerocercoids of D. osmeri and D. ursi are similar, but the former has a much thicker cuticle which is covered with much longer bristles, and the longitudinal subcuticular muscle fibres are arranged in more than one layer (in one layer in D. ursi).

The strobilate stages are dissimilar with respect to size (up to 11 meters by 23 mm. in D. ursi), the distribution of the vitellaria and the testes (mainly in lateral disconnected fields in D. ursi), and in other details of anatomy.

The plerocercoids of D. dalliae are unlike that of D. osmeri in external features and in several details of histology; but their strobilate stages are similar especially as regards the distribution of the vitellaria and the testes, the shape of the uterus, and the relationship of the anteriormost pair of uterine loops to the cirrus pouch.

With the exception of the arrangement of the longitudinal parenchymal muscle fibres, the plerocercoids of D. osmeri and D. cordiceps are entirely different in histology as well as in external features. There is no similarity between their strobilate stages.

D. osmeri resembles D. ditremum in the distribution of the vitellaria and the testes, and in the arrangement and state of development of the longitudinal parenchymal musculature,

but the strobila of D. osmeri is usually much smaller, and the usual shape of the proglottids, the uterus, and the scolex, as well as their plerocercoids, are different.

The plerocercoids of D. osmeri and D. sebago differ in external features (body of D. sebago flattened and with transverse folds which simulate external strobilization), in the absence of frontal glands in the scolex of D. sebago, and in the arrangement of the longitudinal subcuticular muscle fibres (in a single layer in D. sebago). Their strobilate stages resemble to some extent, but differ in the distribution of the vitellaria and the testes in so far as there is a free space anterior to the cirrus pouch in D. sebago.

Apart from the plerocercoid designated D. ditremum by Hickey and Harris (1947), but which bears an external resemblance to D. osmeri, the only other plerocercoids with which D. osmeri may be similar, is that of D. laruei and the Diphyllbothrium larva reported by Cooper (1921) from the red Canadian trout (Salvelinus marstoni). Except for a figure of the scolex and the immediately adjacent portion of the forebody, as well as a brief note on the size and shape of the worm, no other data were supplied by Cooper for this larva whose anterior portion resembles that of D. osmeri. From Vergeer's (1942) limited description of the plerocercoid of D. laruei and from his Figs. 7 and 8 of the scolex and

anterior portion of the body, it would appear that some similarities in external features exist between this worm and the plerocercoid of D. osmeri. However, the data available in these last two cases cited, are too inadequate for proper comparison.

Diphyllbothrium cordiceps (Leidy, 1872) Meggitt, 1924.

Synonymy: Dibothrium cordiceps Leidy, 1872.

(i) The adult stage - Figs. 17 and 18

Strobila large and rugose with moderately dentated lateral borders; 58 to 362 cm. in length by 8 to 12 mm. in greatest width.

Most proglottids many times wider than long with width-length ratio of up to 7:1 in largest proglottids, but 1:2 in posterior portions of strobila; shape varies from slightly trapezoid, through rectangular and square, to oblong; lateral margins of proglottids rectilinear, convex in oblong proglottids of posterior portion.

In fully mature segments, uterus as a treelike spiral of 6-7 pairs of uterine loops loosely arranged and well-separated from one another; up to 9 pairs in posterior segments; proximal 3-4 pairs contain unripe eggs, anterior-most pair sacculate, usually extends no further than half way along lateral borders of cirrus pouch, but sometimes reaches up to and beyond anterior margin of same which then appears buried between them; loops of unequal length with middle pair often much longer than others. Width-length ratio of complex 6:5; occupies central $\frac{2}{5}$ to $\frac{1}{3}$ of proglottid.

Ovary situated in posterior $\frac{1}{3}$ of proglottid; comprised of irregularly arranged cord-like units, or may sometimes

have granulo-recticular appearance; extends laterally well beyond lateral extension of uterine loops, and anteriorly up to anteriormost pair of loops with unripe eggs; lateral lobes joined by narrow transverse portion; anterior horns poorly developed; posterior projections extend into adjacent segment, and often meet, thus surrounding crescent-shape Mehlis gland. Width-length ratio 5:2.

Vitellaria numerous, variable in size and shape but mainly spherical or ovoid, $51 \times 44 \mu$ to $72 \times 58 \mu$ - mode $58 \times 58 - 72 \times 51 \mu$; follicles comprised of several cells; distributed in dorsal and ventral cortex in lateral fields which overlap uterine loops, but never cross mid-line. Vagina large and weakly sinuous; runs anteriorly ventral to uterus, turning dorsad in vicinity of genital atrium and continuing up to mid-point of posterior border of cirrus pouch, then turns back travelling ventrad to open into posterior floor of genital atrium.

Testes variable in size and shape: mainly ovoid with longitudinal axes horizontal, but also pyriform and spherical; distributed in medullary layer, sometimes in more than one layer; in two lateral fields which may have a single row crossing mid-line anterior to cirrus pouch, or more usually not crossing mid-line; occasionally, single row extends across mid-line posterior to ovary; contiguous with tips of uterine loops, but often overlap same and sometimes found between them; arranged

in continuous fields from proglottid to proglottid; 116-276 by 102 to 246 μ (usually 160-203 μ by 131-160 μ).

Cirrus pouch muscular, situated in anterior 1/5 to 1/6 of segment - very close to anterior border of proglottid, anterior boundary often coinciding with that of proglottid; shape and size as seen in sagittal sections very variable even in adjacent proglottids: ovate, pyriform, spherical, top-shaped; extends from half way to three-fourths across medulla, measures 290-508 μ by 188 to 450 μ .

Ejaculatory duct enters dorsal wall from large ovoid external seminal vesicle which receives large vas deferens which follows tortuous course dorsal to uterus from origin near ovarian isthmus. Genital pore opens in ventral mid-line in anterior 1/5 of segment; uterine pore opens short distance posteriorly and slightly to one side of mid-line.

Muscle layers well-developed: in transverse sections, longitudinal fibres arranged in bundles, comprise deep layer, dorsal layer often deeper than ventral; ratio of thickness of outer cortex: inner cortex: muscle layers: medulla = 1:1:1:2 or 2.5. Cortico-medullary ratio = 1:1.

Neck region long and distinctly unsegmented, usually several mm. in length. Scolex mainly ovate or lanceolate and measures from 2.0 - 2.3 mm. by .75 to .9 mm. (mode 2 mm. x .90 mm).

Hosts:

Natural - unknown.

Experimental - dog (100% of 12 feedings), cat
(80% of 10 feedings).

(ii) The plerocercoid stage - Fig. 19

External features

Long, ivory-white worm; 1.0 to 15 cm. in length by 0.5 to 1.3 mm. broad, with widest region in posterior portion of body - mode 6 to 7.5 by 1.0 mm. Body only moderately wrinkled, mainly in anterior portion; rest of body smooth but bears noticeable transverse striations which mimic segmentation. Posterior extremity rounded. Scolex large, olivate to ovoid-lanceolate in dorso-ventral view, somewhat laterally compressed and flattened at apex. Always everted, 1.5 to 2.5 mm. in length by 1.0 mm. in width - mode 2.0 by 1.0. Deep bothria extend length of scolex, becoming wider anteriorly, and open at summit.

Internal structures

Section through scolex ovoid to roundish, that through body ovoid and somewhat dorsoventrally flattened. Cuticle 25-30 μ thick and lacks bristles. Frontal glands poorly developed: scattered throughout scolex and anterior portion of body. Subcuticular muscle fibres in single narrow layer about 4-8 μ wide. Layer of subcuticular cells much wider,

from 28 to 40 μ wide. Intermediate zone from 43 to 55 μ wide. Central zone twice as wide, 90 to 110 μ wide. Parenchymal musculature well-developed: longitudinal fibres form layer 90 μ thick; fibres coalesced into large bundles, and in many cases seem somewhat diagonally arranged. Transverse fibres in a layer from 15 to 50 μ thick. Pair of longitudinal nerve cords and main pair of excretory ducts in central zone. Numerous peripheral excretory ducts perforate section.

Host: rainbow trout.

Location in host: unencysted, but occasionally encysted, on or in viscera; encysted in muscle, mainly hypaxial, in region of stomach.

(iii) The coracidium - Fig. 25

Hatches in 6-8 days when egg incubated at 20°C.; tumbling, spiro-rotary movement accomplished by means of ciliary action; at room temperature, decrease in activity after 25-30 hours, immobility and death by 45 to 50 hours. Somewhat ovoidal when hatched, becoming spherical shortly afterwards, 54 to 72 μ in diameter when hatched, increasing to more than twice original size within hours.

Ciliated embryophore up to 14 μ thick surrounds spherical oncosphere. Cilia at anterior pole up to 44 μ long, up to 18 to 20 μ at posterior end. Three pairs of hooklets at posterior end of oncosphere, 12.5 to 13.5 μ long, middle pair

longer than lateral ones; ratio of blade length to length of handle 1:3.

(iv) The egg - Fig. 26

Amber-coloured, usually ovoidal or somewhat pyriform in shape; egg-shell smooth, about 1.2 - 1.5 μ thick, operculum at one end, boss sometimes present at other end; 58 - 72.5 by 43-51 μ , usually 65 by 46 μ in size. Unembryonated when discharged, ovum hidden from view by vitelline cells. At 20°C., hexacanth becomes completely visible by fourth day, fully developed and ready for hatching by sixth to eighth day; if incubated in darkness, would often hatch before exposure to light. Retains ability to hatch for more than a year when stored at 4°C.

(v) Morphological relationships

Much confusion and indecision exist over the correct systematic position of Diphyllobthrium (= Dibothrium) cordiceps, originally described as a plerocercoid from brook trout (Salvelinus fontinalis) in Yellowstone Lake, Yellowstone National Park, Wyoming, and whose adult stage has been reported as a parasite of white pelicans, gulls, and bears in the same locality (Linton, 1891b; Skinker, 1932; Woodbury, 1935; Scott, 1935). Skinker (1932) could find no characters on the basis of which to distinguish between D. cordiceps and D. latum.

She therefore concluded that "on the basis of morphology of the material available for study, D. cordiceps must be considered a synonym of D. latum". Simultaneously, Scott, (1932) reported that the diphyllbothriids recovered from bears in Yellowstone Park (presumed to be D. cordiceps), were D. cordatum, but that the ova and the plerocercoids were identical with those of D. latum. He subsequently modified this view (Scott, 1935), suggesting instead that "we have here a highly variable species with several sub-species, or possibly three closely related species, between which hybridization may occur, all different from D. latum or D. cordatum". After obtaining negative results from attempts to infect himself and dogs with plerocercoids from Yellowstone trout, Woodbury (1935) concluded that the species in question was probably neither D. latum nor D. cordatum but possibly a physiological species distinct from both. Kuhlow (1953b) was reluctant to accept Markowski's (1949) decision to reduce D. cordiceps to a synonym of D. dendriticum, and so expressed the necessity for further study of the matter. And both Vik (1957) and Fraser (1960c) implied that D. cordiceps should be considered a nomen nudum since details of the internal anatomy of the worm are lacking.

Indeed, identification of the adult from the literature is impossible. No figure of the strobila (or of the plerocercoid

stage) accompanied the sketchy descriptions of Scott (1955), in which no mention was made of many anatomical features normally used in classification; and as Meyer and Robinson (1963) have pointed out, the figure of a proglottid included in Linton (1891b) is of little value for comparative purposes.

Rausch (1954), after expressing the view that the only fairly complete description of D. cordiceps, that of Ward (1918), had "no value for taxonomic purposes", went on to emphasize the need for conducting feeding experiments with the two larval forms described as D. cordiceps by Linton (1891a,b), in order to determine which is the larva of D. cordiceps.

From the results of such experiments conducted in the present study, it is clear that only the large larva described and figured by Linton belongs to D. cordiceps. Adult worms raised from larvae similar to the small one pictured by him, were identified as D. ditremum. Those raised in dogs and cats from the large plerocercoid generally fit the limited description of the species and correspond in almost every respect to Rausch's Fig. 9 of a mature proglottid from a Yellowstone Park bear, designated as D. cordiceps (Rausch, 1954). Direct comparison with a stained whole-mount of fully mature proglottids of a specimen obtained from a bear from Yellowstone National Park*,

*Kindly loaned by Dr. Robert Rausch, Arctic Health Research Inst., Anchorage, Alaska. This specimen might have been the basis of his Fig. 9 (1954).

confirmed the similarity. On the basis of the comparison of the plerocercoid with the description and figure of Linton, and of the adults reared in experimental hosts with those from Yellowstone Park bears, the author was able to identify this species as D. cordiceps, a species separate and distinct from those with which it might easily be confused, either in the strobilate or the plerocercoid stage, namely, D. dendriticum, D. norvegicum, D. medium, D. sebago, D. cordatum, Diphyllbothrium sp. (Duguid and Sheppard, 1944), and D. latum.

D. cordiceps resembles D. dendriticum in having a relatively small, more or less ellipsoidal scolex followed by a well-defined neck region; elongate proglottids in the posterior portion of the strobila; ovoid testes with their long axes along the horizontal axis of the proglottid; and an area free of vitelline follicles and testes anterior to the cirrus pouch (this area is often very limited in extent in D. cordiceps).

They differ in the following respects:

1. Size of strobila - in material raised under comparable conditions, strobila of D. cordiceps usually larger.
2. General appearance of strobila - in D. cordiceps, lateral margins only slightly serrated; usually markedly so in D. dendriticum.

3. Shape of proglottids - in D. cordiceps, those of central portion of strobila rectangular and from 4-7 times as broad as long; in D. dendriticum those from comparable portion of strobila rectangular, quadrate or sometimes trapezoid, with width-length ratio of up to 3:1; lateral edges of proglottids, especially of posterior part of strobila, rectilinear or slightly convex in D. cordiceps, concave in D. dendriticum.

4. Multiplicity of genital organs per proglottid - tandem multiplication of genital organs in posterior proglottids very frequent in D. dendriticum; absent in D. cordiceps.

5. Shape of uterus - more tree-like or spiral in D. cordiceps, rosette-like with an overall roundish to ovoid contour in D. dendriticum; loops in D. cordiceps generally clearly separated, in gravid segments of D. dendriticum, more anterior loops often fused, making it virtually impossible to determine their exact number; and lateral extension of loops greater in D. cordiceps than in D. dendriticum.

6. Position of cirrus pouch in most segments - very close to anterior border in D. cordiceps, variable but generally further removed in D. dendriticum.

7. Relative size of testes, and their distribution - mean size and upper limit of range greater in D. cordiceps. Often arranged in separated lateral fields and overlapping

uterine loops in D. cordiceps, more often confluent anterior to cirrus pouch in D. dendriticum but seldom overlapping tips of uterine loops.

8. Distribution of vitellaria - in D. cordiceps, overlapping uterine loops but never crossing mid-line, therefore in separated lateral fields; in D. dendriticum, only occasionally overlapping tips of loops and generally confluent anterior to cirrus pouch.

9. Structure of ovary - mainly of cord-like units in D. cordiceps, granulo-reticular in D. dendriticum for the most part.

10. The overall shape, and details of morphology and histology of their plerocercoids - body of D. cordiceps less wrinkled, and more dorso-ventrally flattened and translucent than that of D. dendriticum; cuticle lacks bristles, and longitudinal parenchymal muscle fibres in distinct fascicles in D. cordiceps.

Since in this study, D. norvegicum is considered a synonym of D. dendriticum, the similarities and differences which exist between D. cordiceps and D. norvegicum should be the same as discussed in the preceeding paragraphs. However, Vik (1957) stated that "the only worm with which D. norvegicum may be identical, based on the appearance of the plerocercoid, is D. cordiceps". But he discounted this possibility because of differences in the shape of the scolex, the absence of

cuticular bristles in D. cordiceps, differences in the site of the plerocercoid in its host (intramuscularly as well as in the body cavity in the case of D. cordiceps, never in the muscle in case of D. norvegicum, and differences in infectibility to man (D. norvegicum readily infective to man, D. cordiceps apparently not).

On the basis of comparisons made with plerocercoids and sections of D. norvegicum kindly supplied by the Parasitological Institute, Abo, Finland, as well as with Vik's figures, the external features and histology of D. norvegicum are unlike those of D. cordiceps. But Vik's Fig. 15, Plate VI, of a large segmented stage II plerocercoid, looks very much like large D. cordiceps plerocercoids.

Likewise, the plerocercoid reported by Duguid and Sheppard (1944) as the cause of an epidemic in the trout of South Wales, seems to have an external resemblance to that of D. cordiceps. From the description of the fully relaxed adult worm, it is evident that at least in the shape of the proglottids (length may exceed breadth), this worm differs from D. cordiceps. The presence of only a short neck region is another point of difference. Information given on the internal anatomy both of the plerocercoid and of the adult, was, however, too sketchy to provide a suitable basis for comparison.

Fraser (1960c) included the plerocercoid of D. cordiceps among those which most closely resemble that of D. medium, but since the data given on the plerocercoid of D. medium is so deficient, comparison with D. cordiceps in the present study is not possible.

Although primarily a parasite of pinnipeds, D. cordatum was originally described by Leuckart (1863) from man and dog, and as stated earlier, Scott (1932) identified cestodes from bears in Yellowstone National Park (generally considered to be D. cordiceps) as D. cordatum.

This species resembles D. cordiceps in having short, broad proglottids with well-developed muscle layers. In some D. cordiceps specimens of the present study, the testes were distributed in more than one layer (Fig. 18a), which is considered by Markowski (1952) as a "typical feature of D. cordatum". In other respects, the two species are dissimilar. From Markowski's (1952) description and figures of D. cordatum, this species obviously differs markedly from D. cordiceps in the shape of the scolex (cordate and wider than long in D. cordatum), in the absence of a neck region in D. cordatum, in the number of layers of longitudinal muscle fibres and in the arrangement of these fibres (in D. cordatum, three distinct layers, fibres not in bundles: one layer immediately under cuticle, one immediately external to vitellaria, and a third bordering the medullary parenchyma). The author could not find a description of the

plerocercoid of D. cordatum.

D. cordiceps and D. latum both have large strobilae, proglottids which are wider than long in the central region of the worms; and a well-defined neck region. But they differ with respect to:

1. the width-length ratio of the proglottids;
2. the structure and arrangement of the genital organs, especially the structure of the ovary, and the arrangement and lateral extension of the uterine loops;
3. the shape of the scolex; and
4. their plerocercoids - both species have large plerocercoids with cuticle lacking bristles, but the cuticle of D. latum is more wrinkled; the scolex of D. cordiceps is never invaginated, that of D. latum almost always invaginated; and the longitudinal muscle fibres of D. cordiceps are arranged into well-defined bundles, those of D. latum as individual units.

Diphyllbothrium latum (Linnaeus, 1758) Lühe, 1910.

Synonymy: Taenia lata Linnaeus, 1758.

Bothriocephalus latus of Bremser, 1819.

Dibothriocephalus latus of Lühe, 1899.

Diphyllbothrium latum of Lühe, 1910.

(i) The adult stage - Figs. 20 and 21

Large, ribbon-like strobila of nearly uniform width throughout and slightly serrated margins; 65 cm. to 156 cm. in length by 8 mm. to 10 mm. in greatest width. Proglottids mainly rectangular in shape with width-length ratio of 2:1, and rectilinear lateral borders.

Uterus in fully mature segments a loose spiral of 6-10 loops on each side of mid-line, often asymmetrically arranged; proximal pair of loops contain only unripe eggs; anteriormost pair sac-like, often extends beyond anterior border of cirrus pouch, but usually up to mid-way along pouch or to its anterior border; width-length ratio 1:2. Ovary comprised of cord-like structures forming lattice-work arrangement which surrounds approximately half of uterus, extending up to first pair of loops with ripe eggs; no anterior nor posterior horns present; width-length ratio 11:9. Mehlis gland not easily seen, but half-moon shaped in cases observed.

Vitellaria numerous and of variable shape; follicles large and comprised of many small cells; mainly irregularly spherical

or ovoid, but some reniform; $58 \times 44 \mu$ to $131 \times 87 \mu$ (mode 87×72 to $102 \times 87 \mu$); distributed in dorsal and ventral cortical zones in separated lateral fields contiguous with tips of uterine loops. Vagina a relatively straight tube passing anteriad ventral to uterus; turns dorsad in vicinity of genital atrium running along posterior border of cirrus pouch then turning back to enter posterior wall of genital atrium.

Testes large, mainly ovoid or pyriform, sometimes roughly spherical or reniform; 131 by 131μ to 218 by 138μ with a modal size of $160-189 \mu$ by $116-145 \mu$; arranged in single medullary layer in two separated lateral fields; longitudinal axis oriented along antero-posterior axis of proglottid; co-terminal with tips of uterine loops.

Cirrus pouch large, pyriform and extends completely across medulla when viewed in sagittal section; measures from 580 to 609μ by 406 to 435μ . Ejaculatory duct enters cirrus pouch at postero-dorsal margin after leaving antero-ventral wall of ovoid seminal vesicle which receives large and convoluted vas deferens which passes dorsal to uterus from origin near ovary.

Genital pore opens ventrally in midline in anterior fifth of segment; uterine pore opens short distance posteriorly and to one side of midline. Muscle layers well-developed: in transverse section, longitudinal fibres appear in distinct

and well-separated bundles forming deep layer. Ratio of thickness of outer cortex: inner cortex: muscle layers: medulla =1:2:1:2. Cortico-medullary ratio =2:1. Neck region usually several mm. long. Scolex mainly spatulate, sometimes lanceolate, 2.0 to 3.2 mm. long by 1.0 to 1.2 mm. wide - mode 2.8 mm. by 1.0 mm.

Hosts:

Natural - dog (1957 record, Iosegun Lake); human (one infection in Kootenay Lake district).

Experimental - dog (75% of 4 feedings, and 100% of 2 feedings in 1961), cat (100% of 4 feedings, but all specimens immature), human (one out of one feeding in 1961).

(ii) The plerocercoid stage - Fig. 22

External features

Thick, ivory-white to creamish worm; measures 4 to 50 mm. long by 1 to 2 mm. wide and up to 0.5 mm. thick - mode 10 to 20 mm. by 1.5 mm. by 0.5 mm. Body deeply wrinkled, about same width along length, and bears marked invagination at posterior end. Scolex always invaginated or only slightly evaginated, therefore not well defined. Bothria in some cases represented by small grooves, more often as invagination at anterior end of body.

Internal features

Transverse section through body ovoid. Cuticle 11 to 14 thick, and without bristles. Frontal glands well-developed, extend from invaginated scolex well into second half of body. Subcuticular musculature a single row of fibres. Layer of

sub-cuticular cells narrow but thicker than preceeding layer - about 18 μ thick. Intermediate zone many times wider than sub-cuticular cell layer - about 85-90 μ wide. Longitudinal parenchymal musculature well-developed and comprise a layer about half to two-thirds as thick as intermediate zone - about 36-50 μ thick; fibres not grouped into bundles. Transverse muscle layer weakly developed. Central zone about width of intermediate zone or slightly wider - about 90-110 μ wide, contains lateral nerve cords and pair of slit-like excretory ducts. Several peripheral excretory ducts and many calcareous corpuscles present.

Hosts: northern pike, walleye, and rarely rainbow trout.

Location in host: unencysted in musculature of main hosts; more often in epaxial than in hypaxial muscle; in some cases, unencysted on viscera; unencysted on viscera of rainbow trout.

(iii) The egg - Fig. 26

Light amber in colour, ovoidal or slightly pyriform in shape; egg-shell smooth, operculum at narrower end, no boss seen at opposite pole; measures 63-67 μ by 40-44 μ , usually 64 μ by 44 μ . Unembryonated when shed, ovum completely covered by vitelline cells. When incubated at 20°C., hexacanth becomes fully developed, and hatches by 10th to 12th day (based on observations from a single culture).

Undoubtedly, the most studied species of this genus is D. latum, probably because of its medical importance. In western Canada, the plerocercoid has been reported from northern pike and walleye from Manitoba, Alberta, and the North West Territories (Nicholson, 1928, 1932; Wardle, 1935). The adult worm has been reported from dogs, foxes, and human beings from those localities, and from Saskatchewan (Wardle, 1935; Saunders, 1949; Wolfgang, 1954), and from a bear in British Columbia (Wolfgang, 1954). The only record of human infection in British Columbia, as far as the author is aware, is that reported in the present study. Additional records of plerocercoid and adult infections in Alberta and the North West Territories are listed in Table 2.

Both the plerocercoid and the adult stages fit authentic descriptions of the species - Wardle and McLeod (1952), Kuhlow (1953a), and Wikgren and Muroma (1956). Histological details of the plerocercoid are in accordance with Kuhlow's (1953a) description. Because of its invaginated scolex and wrinkled body, this plerocercoid could be identified with little difficulty, and could be easily differentiated from all other plerocercoids of this genus. Since this species has been so well worked over by other investigators, no further comparison with other species is made herein.

Diphyllobothrium sp. Type I

(i) Plerocercoid - Fig. 23

External features

Slender, somewhat cylindrical worm, white or creamish in colour, 3.0 to 12.0 mm. long by 0.3 to 0.5 mm. broad (mode 6-9 mm. by 0.4 mm.). Unwrinkled body attenuated posteriorly, terminating in sharp point. Scolex olivate; occasionally ovate-lanceolate; always evaginated; about same width as body when viewed in dorso-ventral position; somewhat wider than body in lateral view; 0.5 to 1.0 by 0.3 to 0.5 (mode 8 x 0.5) Bothria extend full length of scolex.

Internal features

Transverse section through scolex and body ovoid. Cuticle 5 to 11 μ thick and covered with bristles of about same length. Frontal glands poorly developed, as few scattered cells confined to scolex. Subcuticular fibres arranged in more than one layer about 7-8 μ deep. Zone of subcuticular cells about 18 μ wide. Intermediate zone much wider, about 28-30 μ in width, and half as wide as central zone with width of about 60 μ . Longitudinal parenchymal muscle layer about same thickness as width of intermediate zone. Circular muscle layer about 11 μ thick. Paired longitudinal nerve cords in central zone and lateral to main pair of excretory ducts. **Large numbers** of refractile calcareous corpuscles present in

all zones.

Host: kokanee, rainbow trout, dolly varden char.

Location in host: in thin-walled cysts or lying free on serosa of stomach, perigastral fat, caeca, gonads and mesentery; only one worm per cyst; cyst spherical or ovoid, 1.5 to 2.0 mm. in greatest dimension.

(ii) Morphological relationships

The only plerocercoids to which Diphyllobothrium sp.

Type I bears an external resemblance are some small D. osmeri plerocercoids, and the "young plerocercoids of D. laruei" (Vergeer 1942, Figs. 9 and 10). Histologically, this species is unlike any other plerocercoid described in the literature, though it bears a slight resemblance to small D. osmeri of this study.

The fact that the feeding experiments with this larva were all negative may imply that these are young forms which are incapable of infecting the definitive hosts. No host has therefore been established for this species.

Diphyllbothrium sp. Type II

(i) Plerocercoid - Fig. 24

External features

Ivory-white worm 8-12 mm. long by 0.6 to 1 mm. at widest part of body (mode 6-8 by 0.8). Moderately wrinkled body tapers posteriorly to a sharp point; dorso-ventrally depressed. Scolex not well delimited from body - separated only by deep transverse furrow; measures 0.7 to 1.0 mm. long and is same width as body. Bothria deep grooves extending full length of scolex and gaping open at summit.

Internal features

Transverse section through scolex roundish, through body ovoid. Cuticle rather thin, 4 to 5.5 μ thick, and covered with bristles 11 to 18 μ long - those on scolex and in bothrial grooves shorter than those on anterior portion of body. Frontal glands as single cells or clumps of few cells scattered throughout scolex especially in its posterior portion and do not extend into body. Subcuticular muscle fibres in single layer 3-4 μ wide. Subcuticular cell layer absent. Intermediate zone wide, about 40 μ . Central zone wider, about 55 μ wide, and containing numerous calcareous corpuscles. Longitudinal parenchymal muscle layer of coarse fibres about 36 μ deep, well-developed. Transverse muscle layer about half as thick, about 18 μ in thickness. Pair of main excretory ducts medial to

lateral nerve cords in central zone.

Hosts: kokanee, rainbow trout, dolly varden char.

Location in host: encysted in thin-walled cysts on serosa of stomach, in perigastral fat, on caeca, and gonads; cysts spherical or ovoid, 1.5 - 2.5 mm. in greatest dimension, and contains only one worm.

(ii) Morphological relationships

Diphyllbothrium sp. Type II somewhat resembles the plerocercoid of D. dendriticum in the wrinkled nature of the cuticle, but the shape of their bodies is different. In histological characteristics, they are alike in having a single layer of subcuticular muscle fibres and in the development of the frontal glands. In other respects, the histology of this species is different from any described in the literature. The large number of calcareous corpuscles seen in transverse sections and the presence of simple glands among the longitudinal muscle fibres of the posterior portion of the scolex and the anterior part of the body, are peculiar to this species (Fig. 26c,d). As in the case of D. sp. Type I, the negative results of feeding experiments with this larva may indicate its incapability to infect the hosts probably because it is a juvenile form.

Important adult characteristics of the species described are summarized in Table 3. Such characteristics of plerocercoids are summarized in Table 4.

Fig. 5 - Explanatory diagrams transverse section through a plerocercoid.

- | | |
|---------------------------------|---|
| A - Through scolex | B - Through body |
| a - bothria | g - longitudinal parenchymal muscle layer |
| b - frontal glands | h - transverse muscle fibres |
| c - cuticle | i - central zone |
| d - subcuticular muscle layer | k - lateral nerve cord |
| e - layer of subcuticular cells | l - main excretory duct |
| f - intermediate zone | |
-

Fig. 6 - Explanatory diagrams of transverse and sagittal sections through a mature proglottid.

- | | |
|-------------------------------|------------------|
| a - outer cortex | f - testis |
| b - inner cortex | g - vitellaria |
| c - longitudinal muscle layer | h - uterus |
| d - transverse muscle fibres | i - ovary |
| e - medulla | k - cirrus pouch |

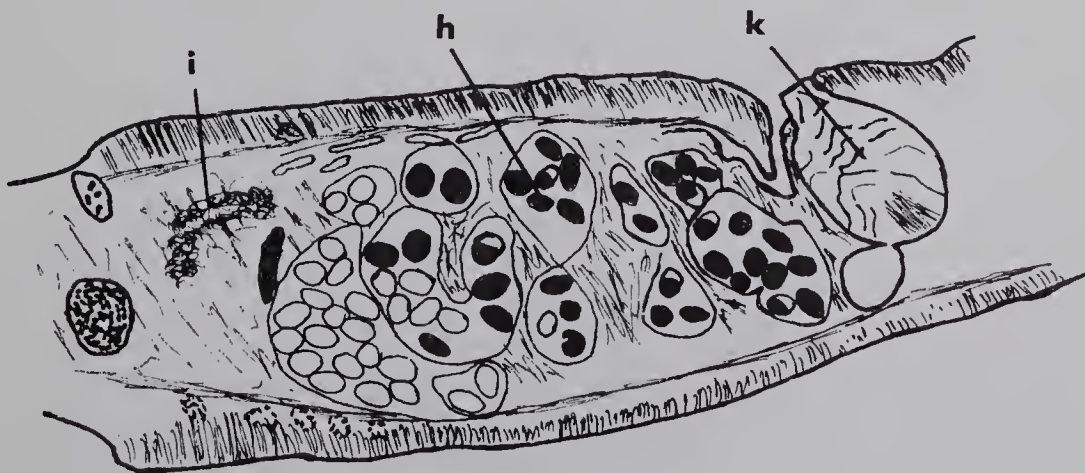
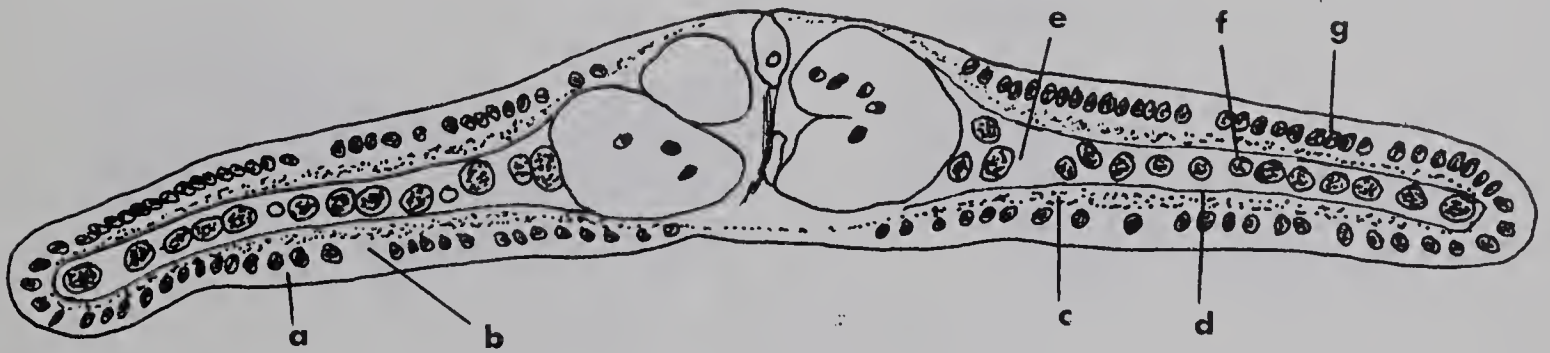
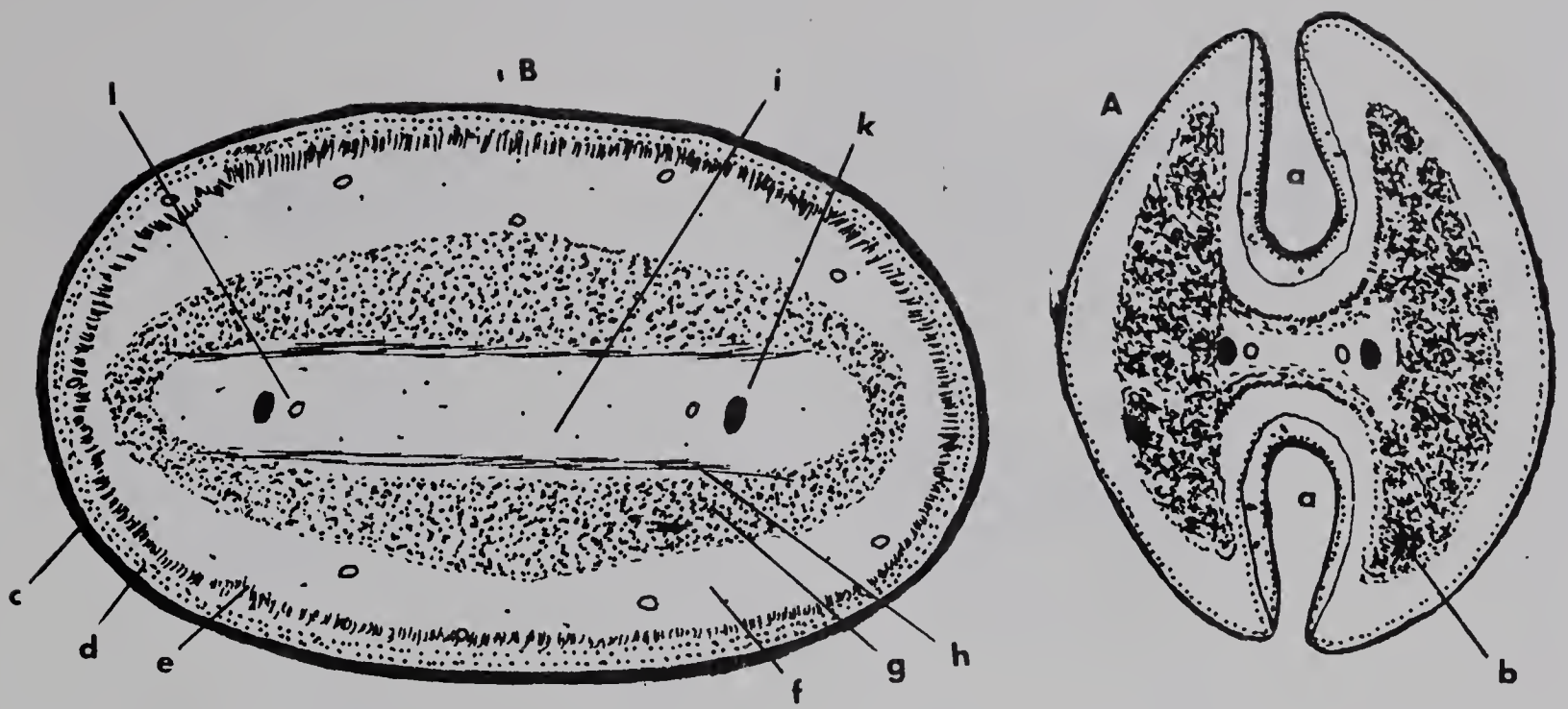


Fig. 7 - Typical shapes and structures of the ovaries of five species of Diphyllbothrium. X40

a - D. dendriticum

b - D. ditremum

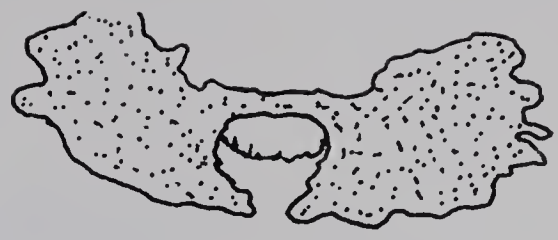
c - D. osmeri

d - D. cordiceps

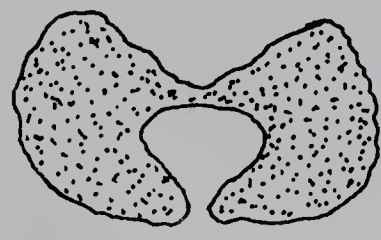
e - D. latum



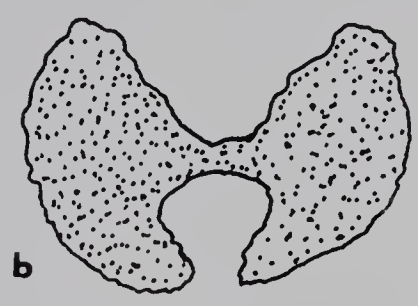
a



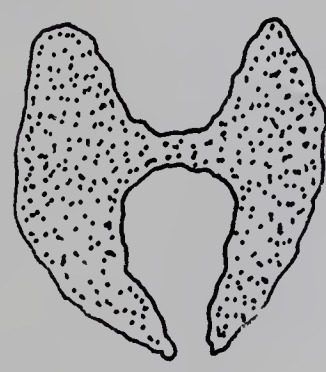
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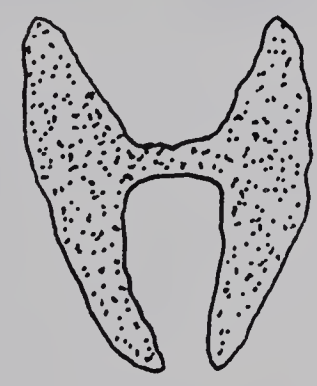
b



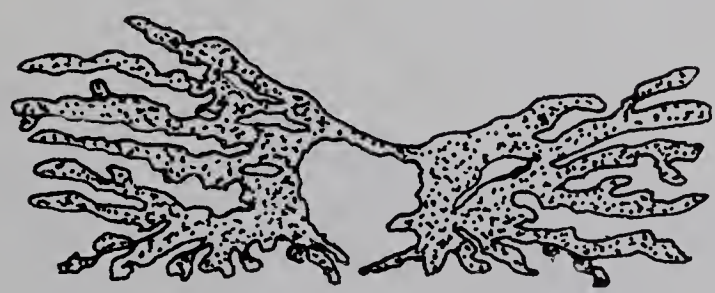
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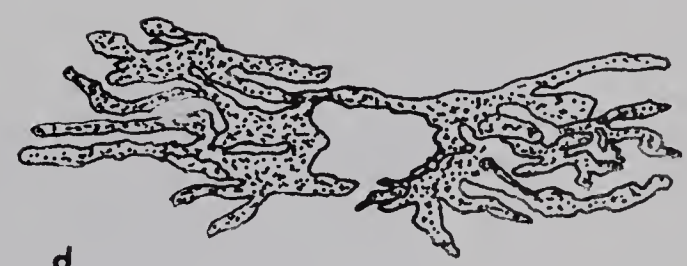
c



c



d



d



e

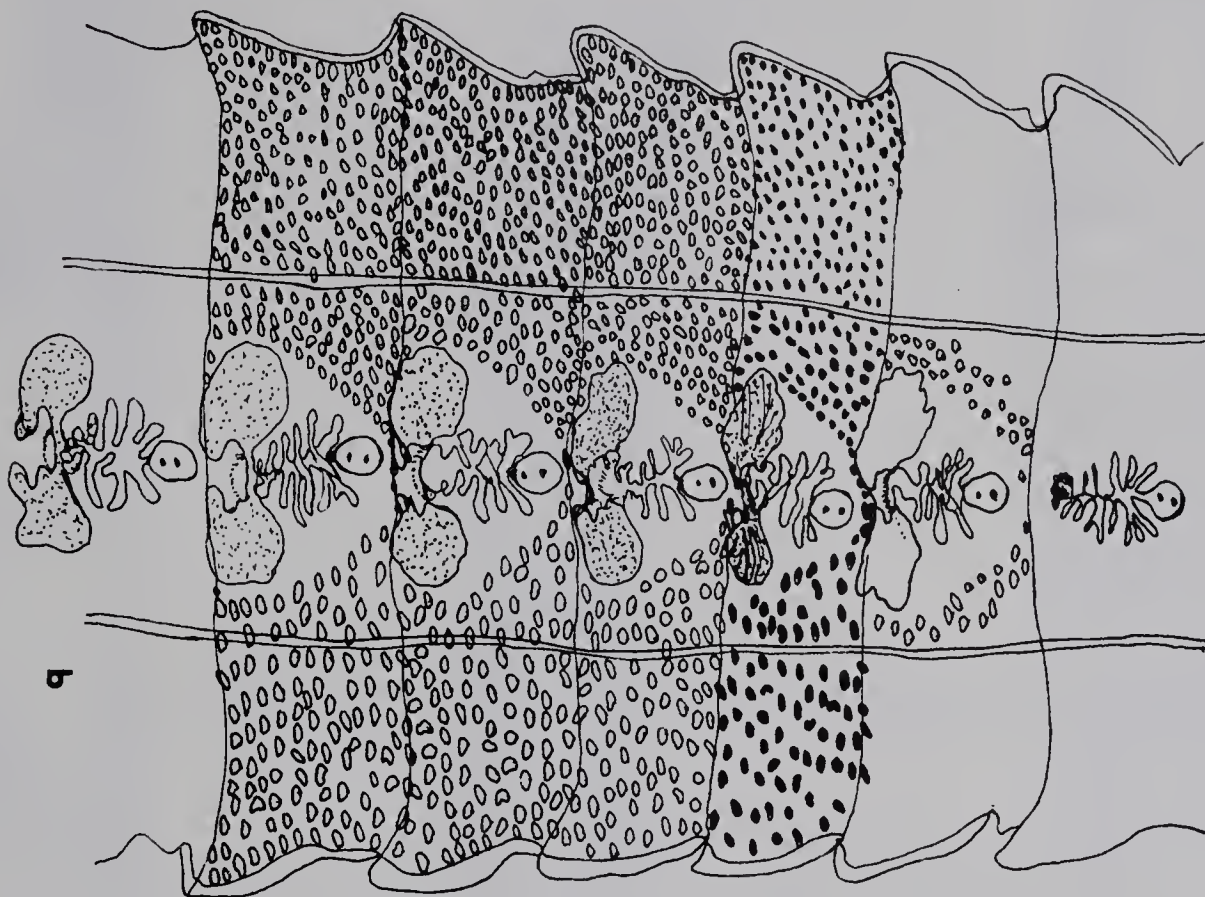
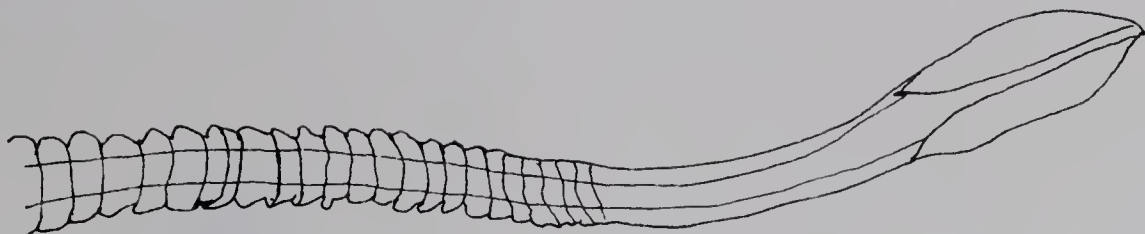
Fig. 8 - D. dendriticum - scolex and proglottids. X12

a - scolex and anterior portion of worm.

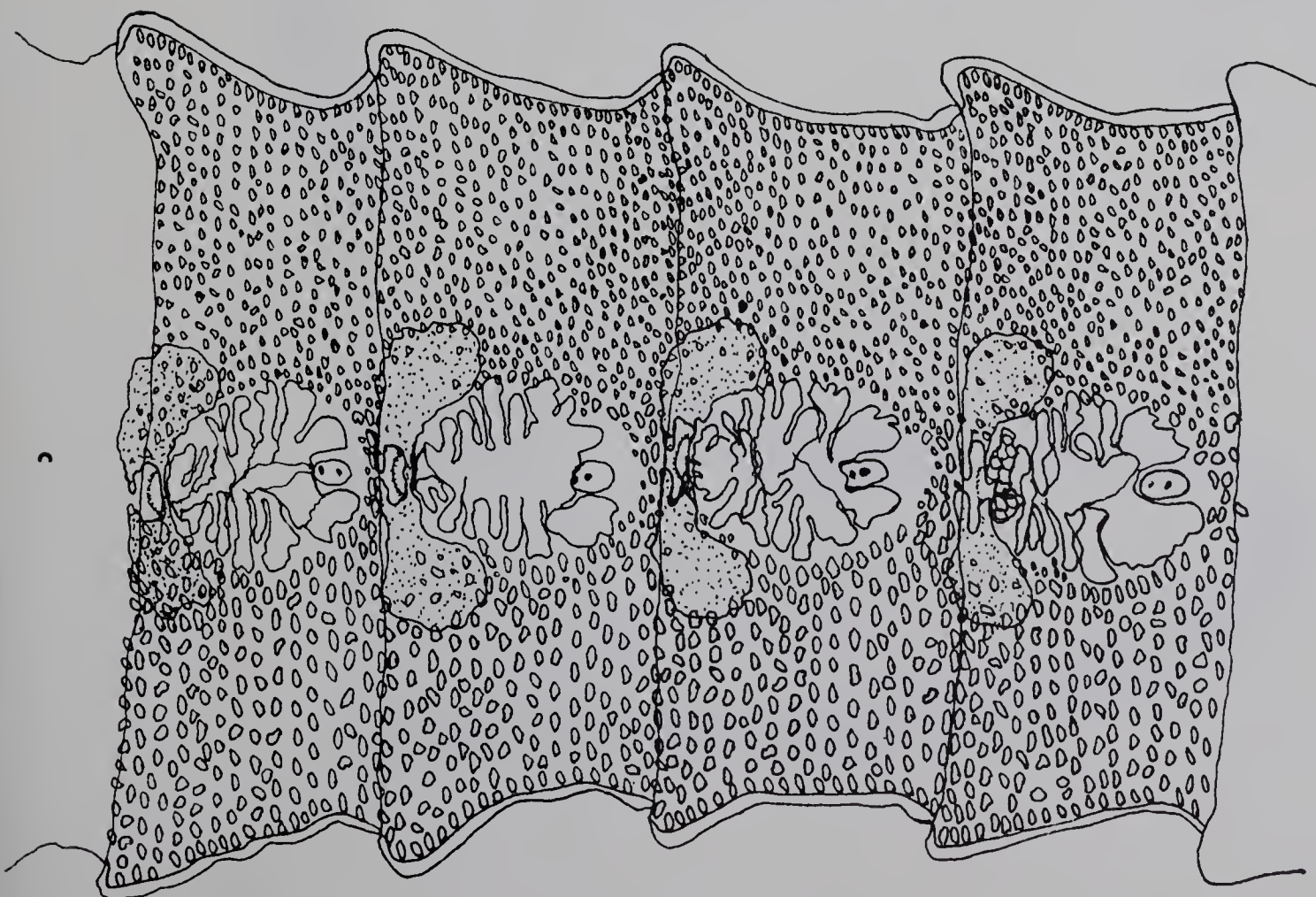
b - early mature proglottids.

c - gravid proglottids.

b



a



c

Fig. 9 - D. dendriticum - sections through mature proglottids. X20.

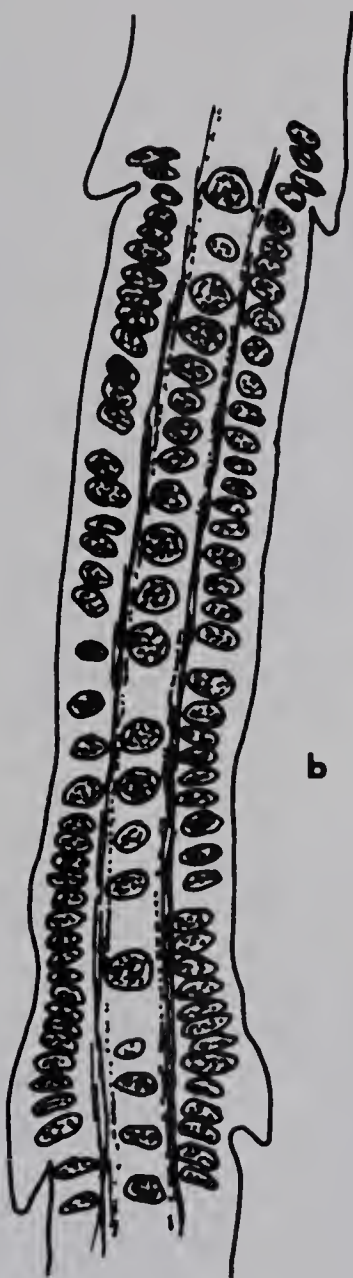
a - transverse section.

b - sagittal section between midline and lateral nerve cord.

c - sagittal section through midline.



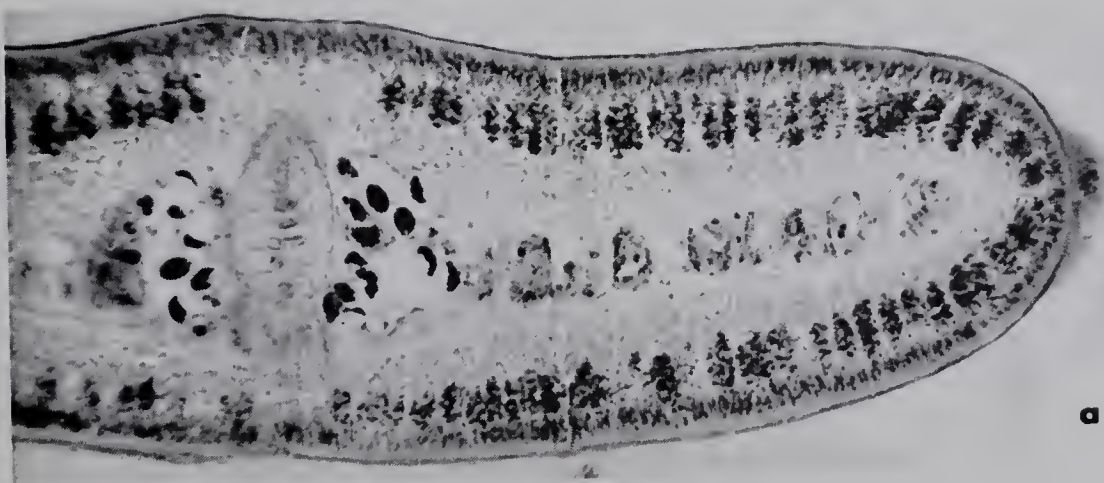
c



b



a



a

Fig. 10 - The plerocercoid of D. dendriticum. X30.

a - anterior portion of worm.

b - terminal portion of worm.

c - transverse section through scolex.

d - sagittal section anterior portion of worm.

e - transverse sections through body of worm.

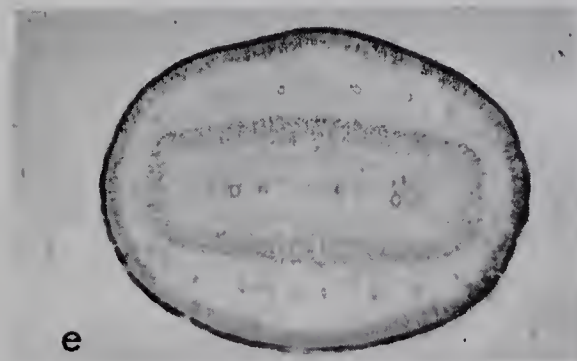
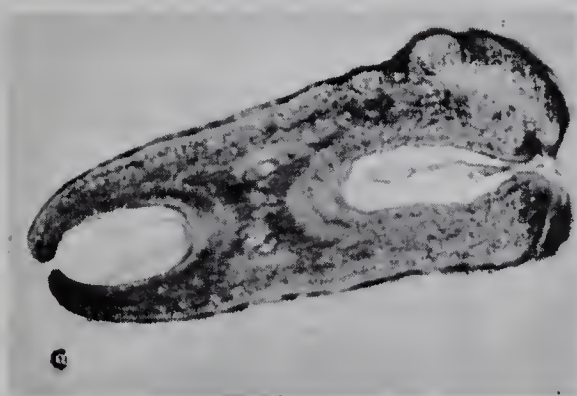
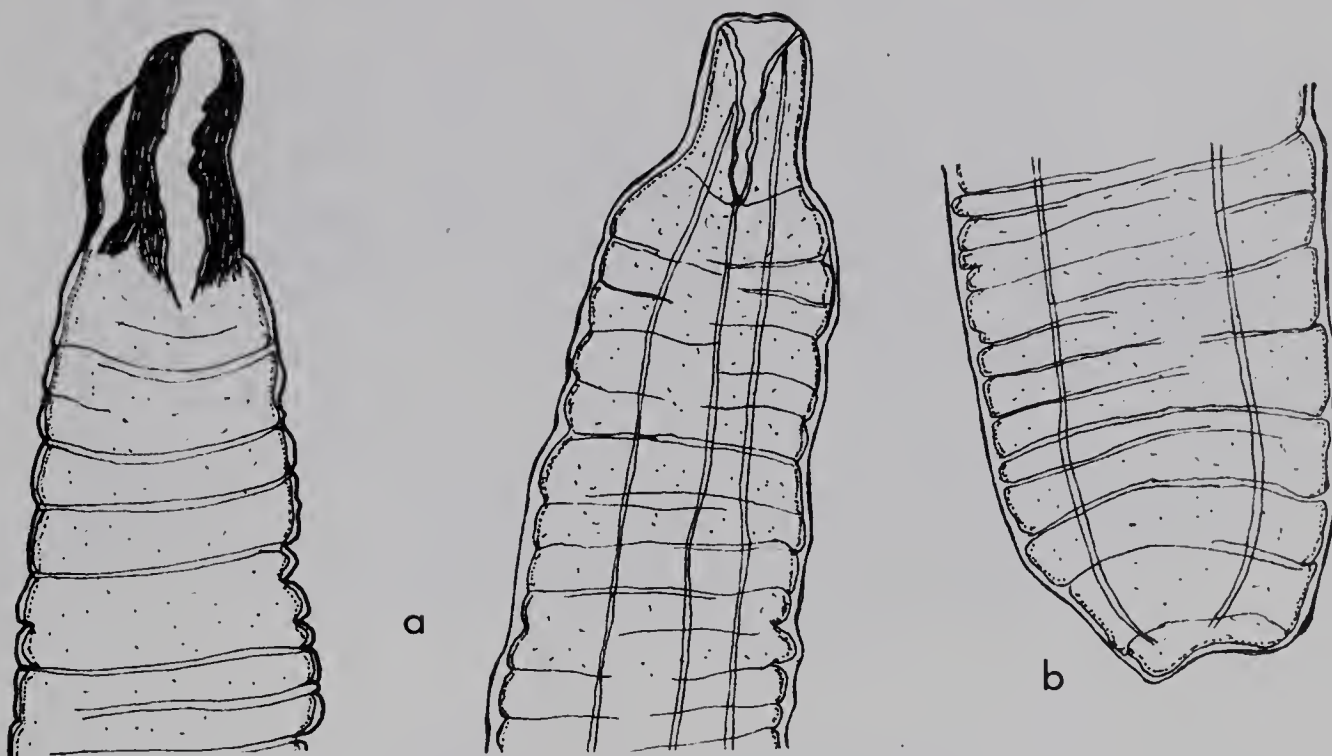


Fig. 11 - D. ditremum - scolex and proglottids. X20.

a - scolex and anterior portion of worm.

b - fully mature proglottids.

c - terminal proglottids.

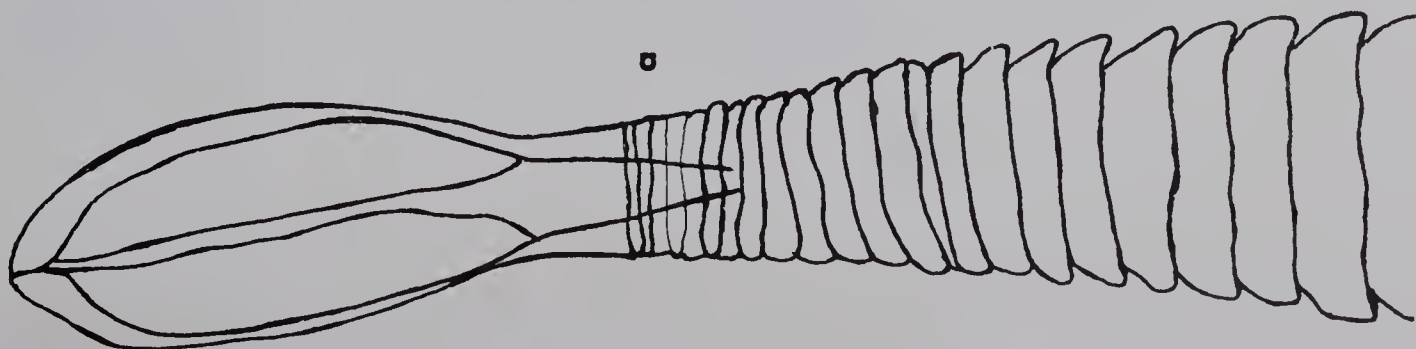
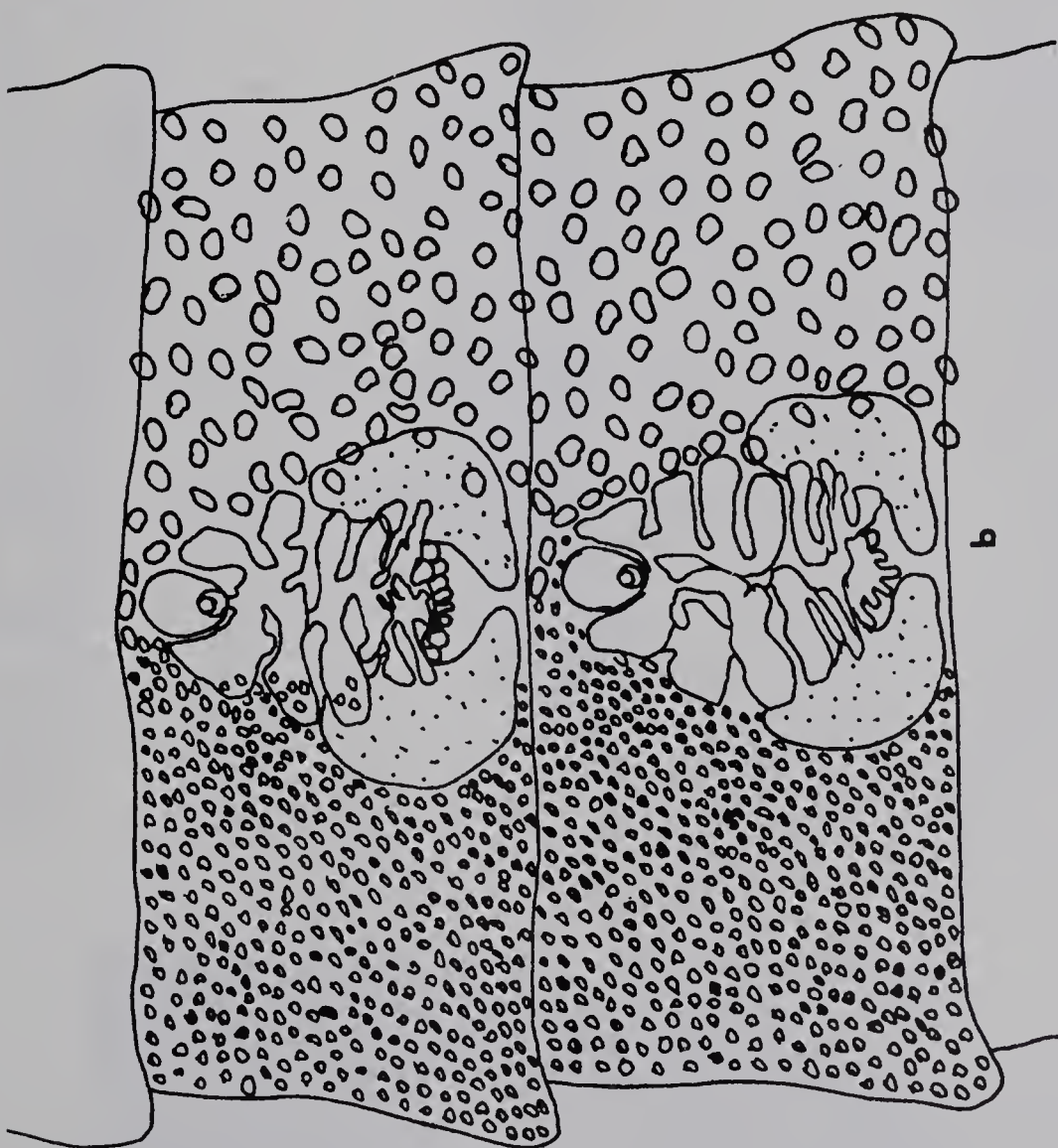
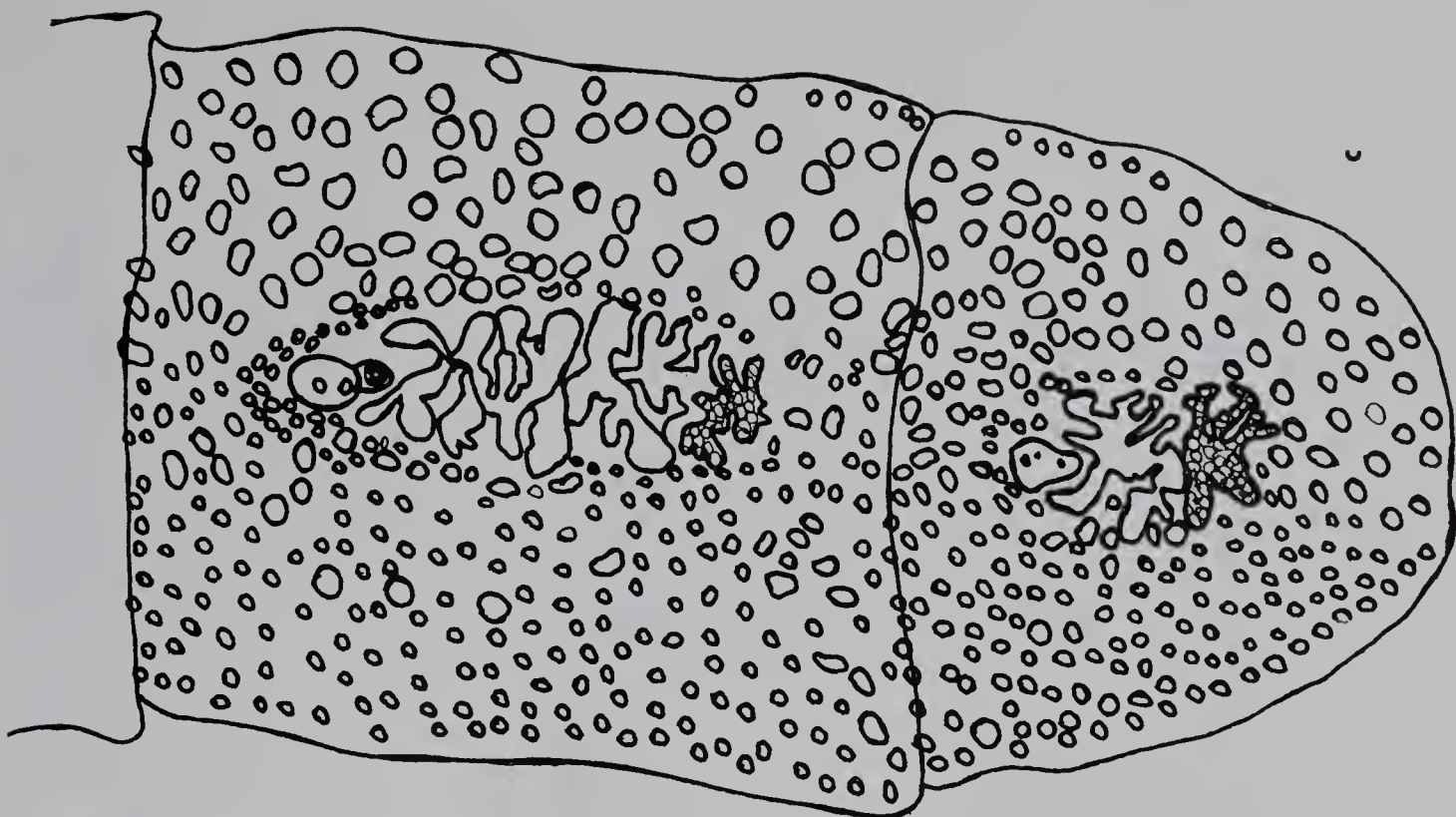


Fig. 12 - D. ditremum - sections through mature proglottids. X30.

a - transverse section.

b - sagittal section between midline and lateral nerve cord.

c - sagittal section through midline.

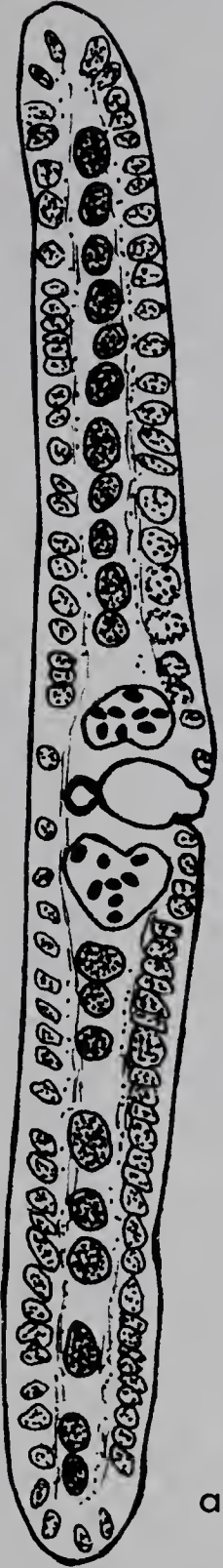
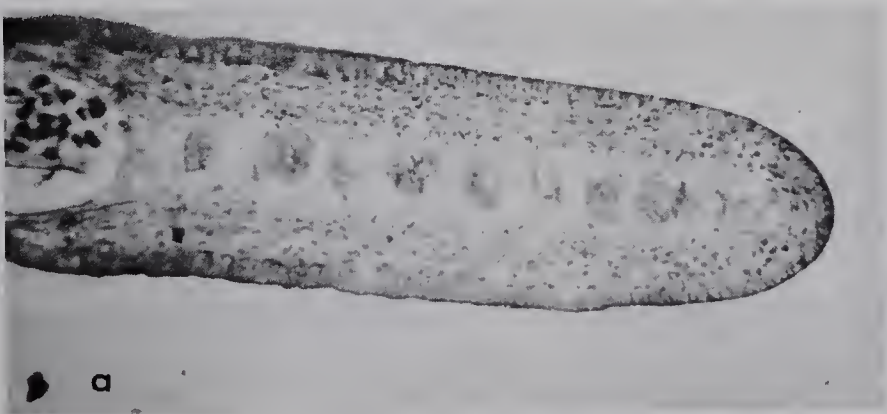


Fig. 13 - The plerocercoid of D. ditremum.

a - complete worms. X10.

b - transverse sections through scolex. X50.

c - transverse sections through body. X50.

d - sagittal section through anterior portion of worm. X40.

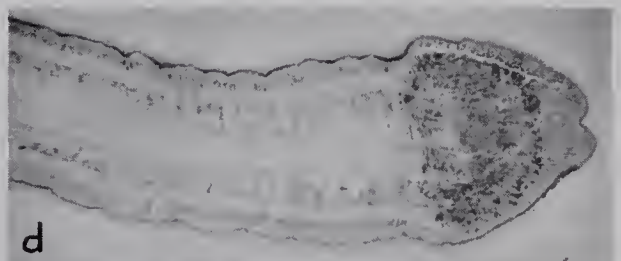
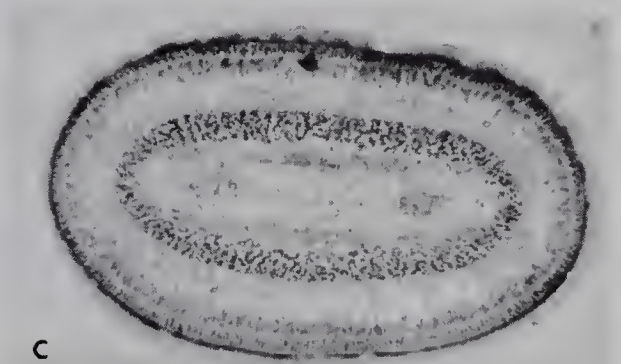
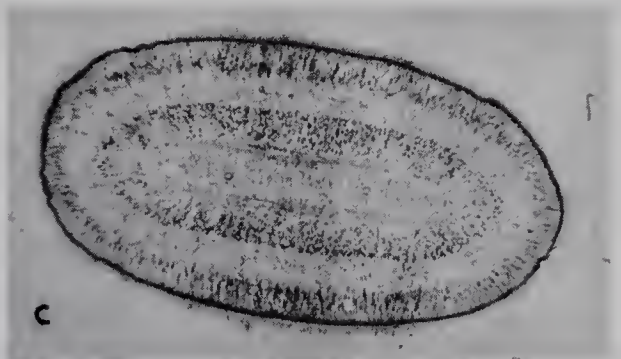
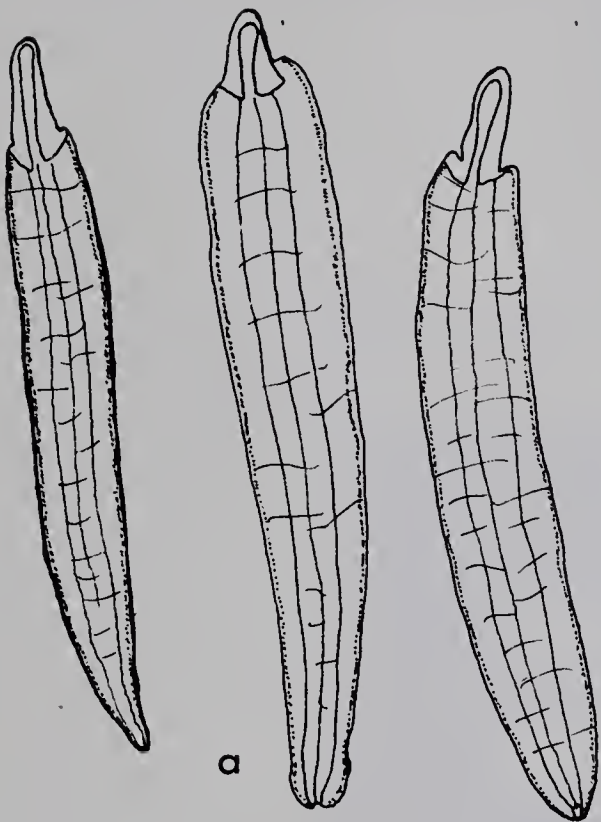
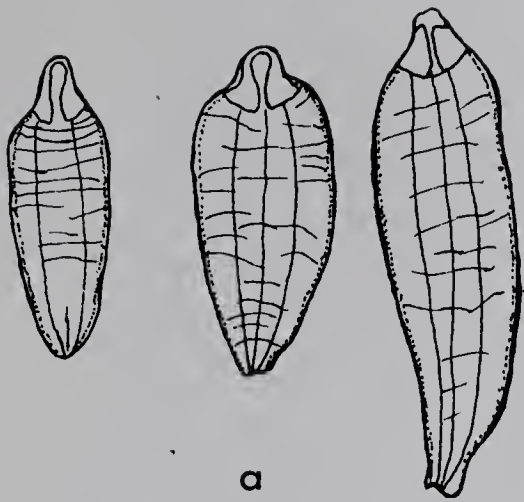


Fig. 14 - D. osmeri - scolex and proglottids. X20.

a - scolex and anterior portion of worm.

b - early mature proglottid.

c - fully mature proglottid.

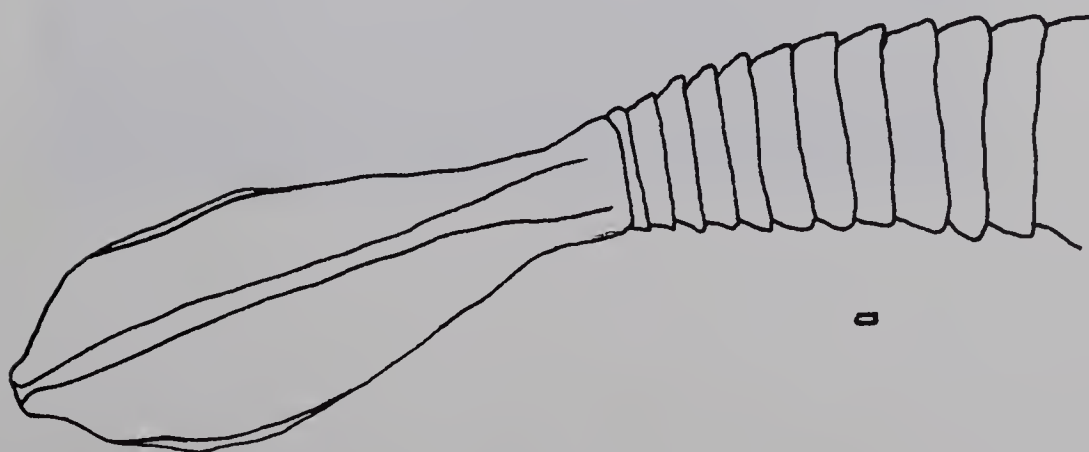
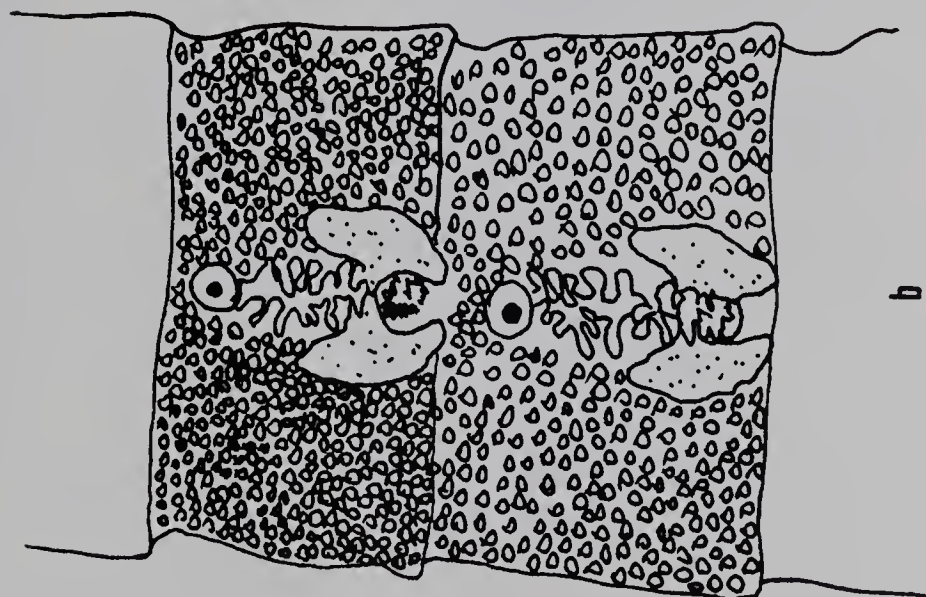
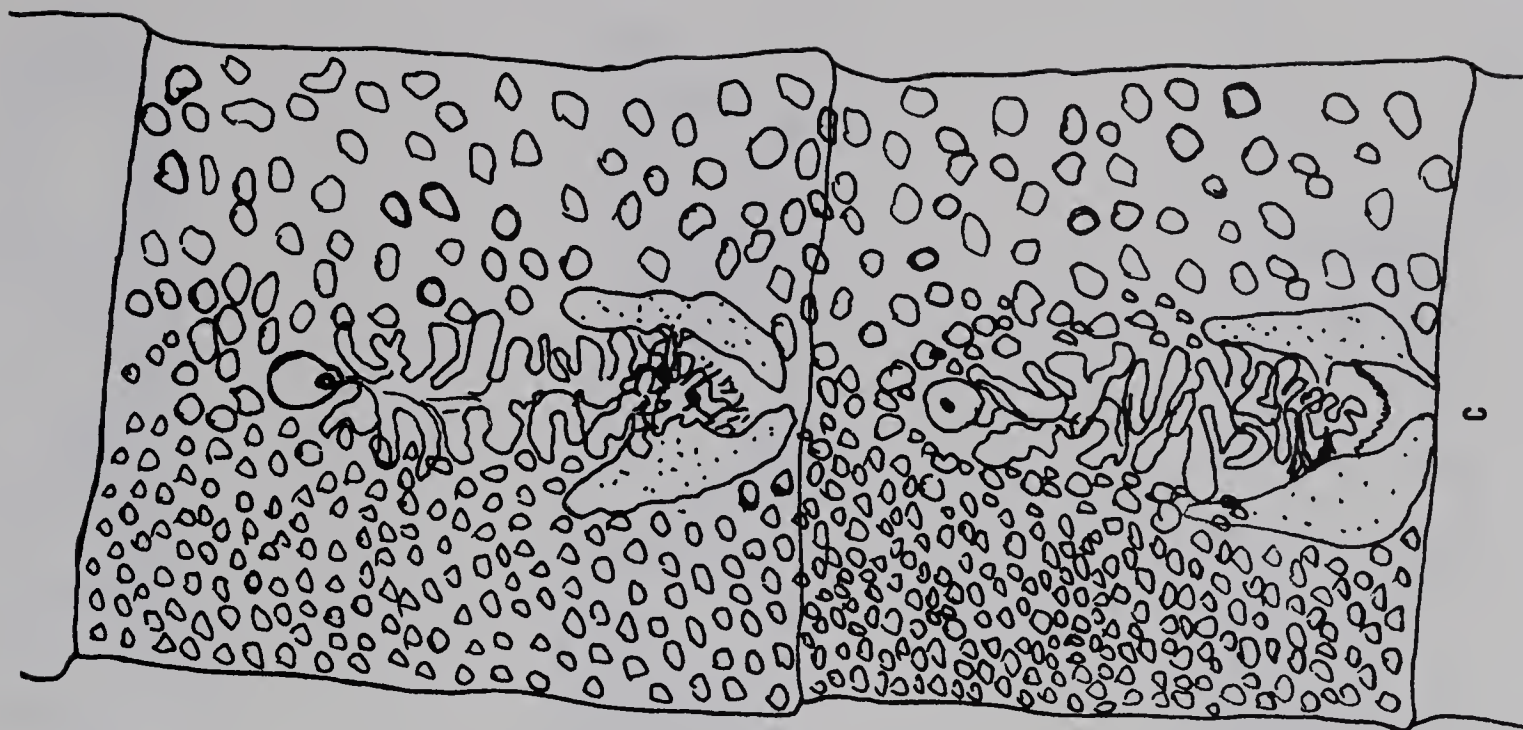


Fig. 15 - D. osmeri - sections through mature proglottids. X25.

a - transverse sections.

b - sagittal section between midline and lateral nerve cord.

c - sagittal section through midline.

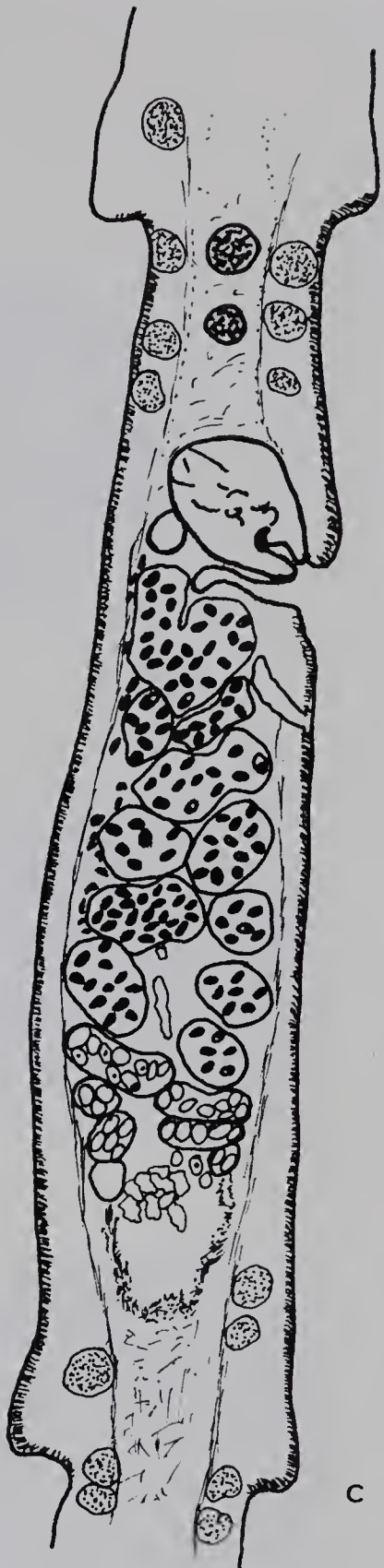
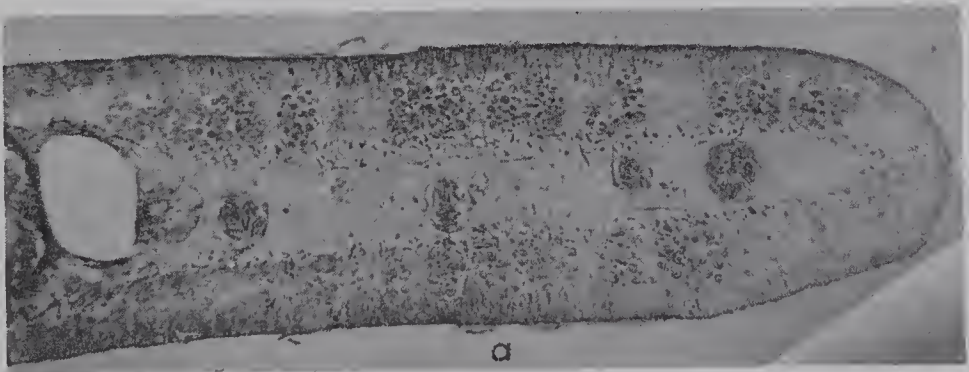
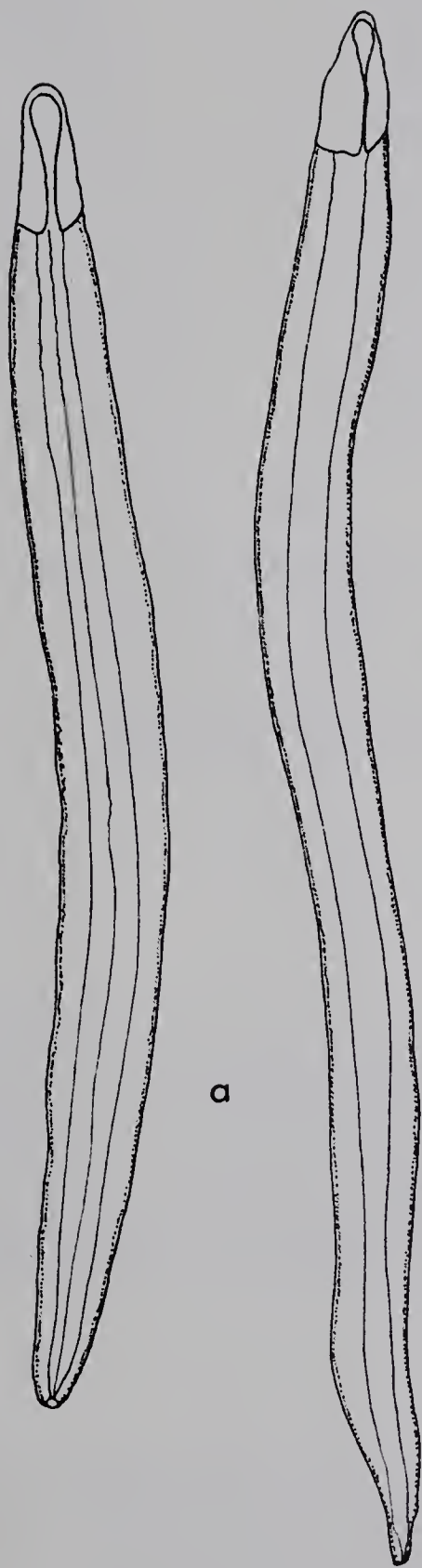
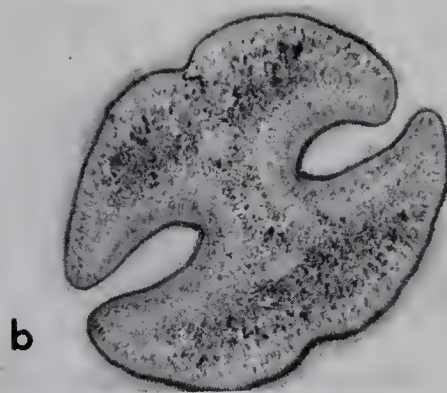


Fig. 16 - The plerocercoid of D. osmeri.

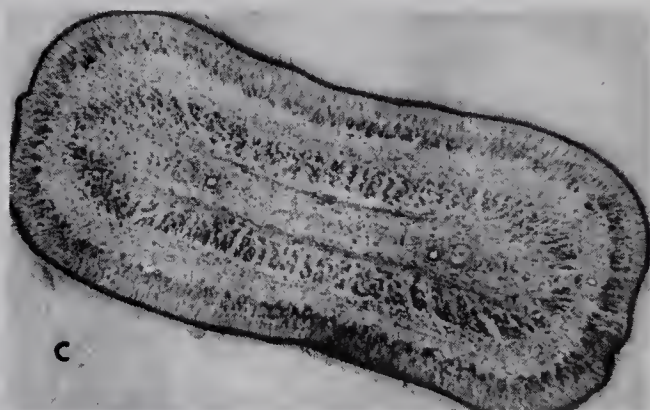
- a - complete worms. X15.
- b - transverse section through scolex. X35.
- c - transverse section through body. X65.
- d - sagittal section through anterior portion of worm. X15.



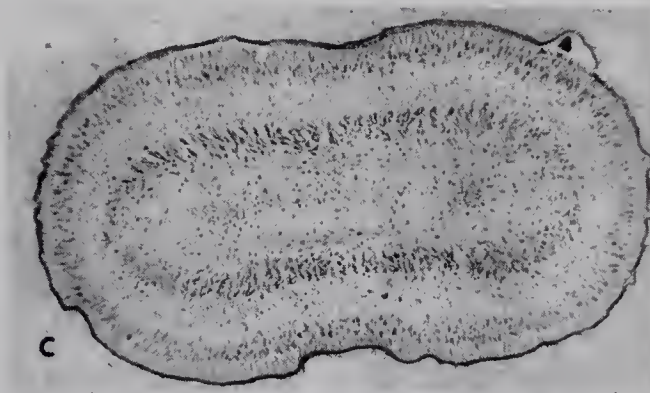
a



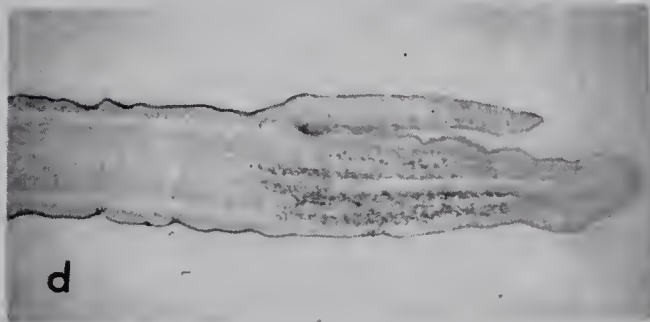
b



c



c



d

Fig. 17 - D. cordiceps - scolex and proglottids. X12.

a - scolex and anterior portion of worm.

b - fully mature proglottids from two
different specimens.

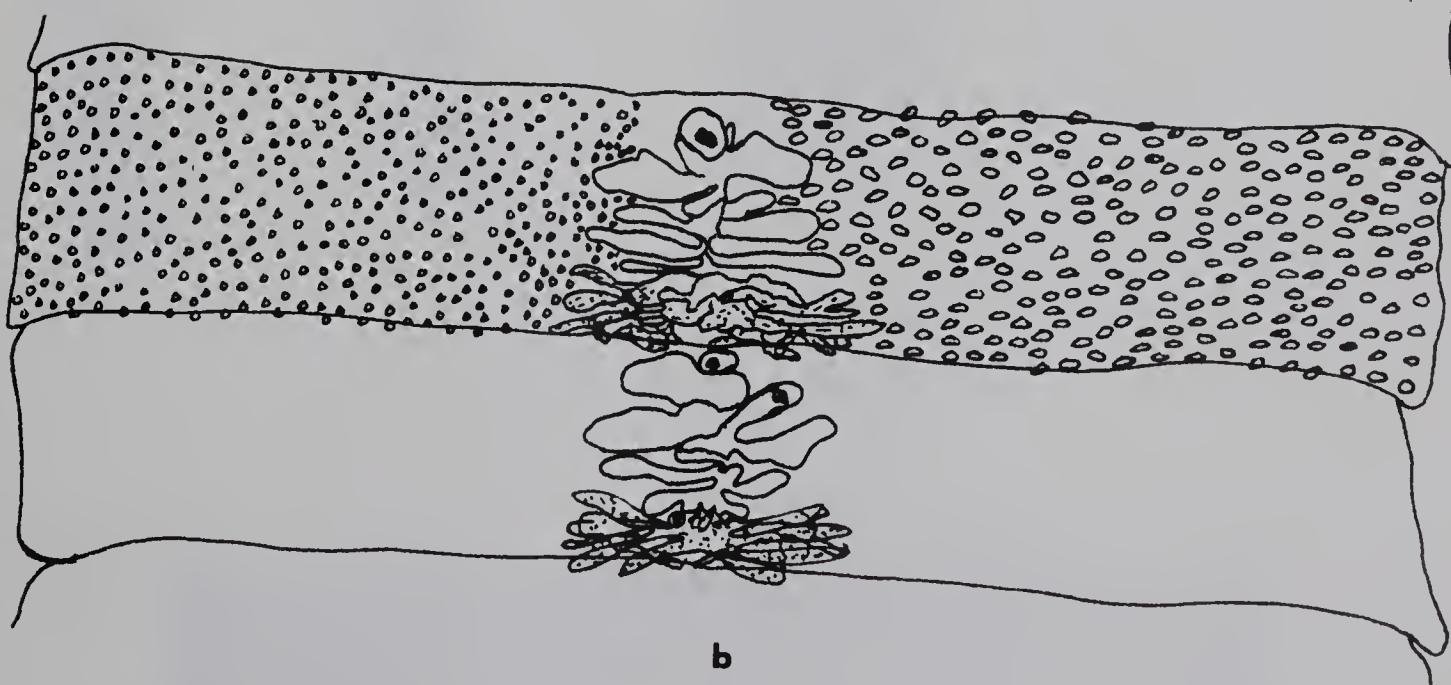
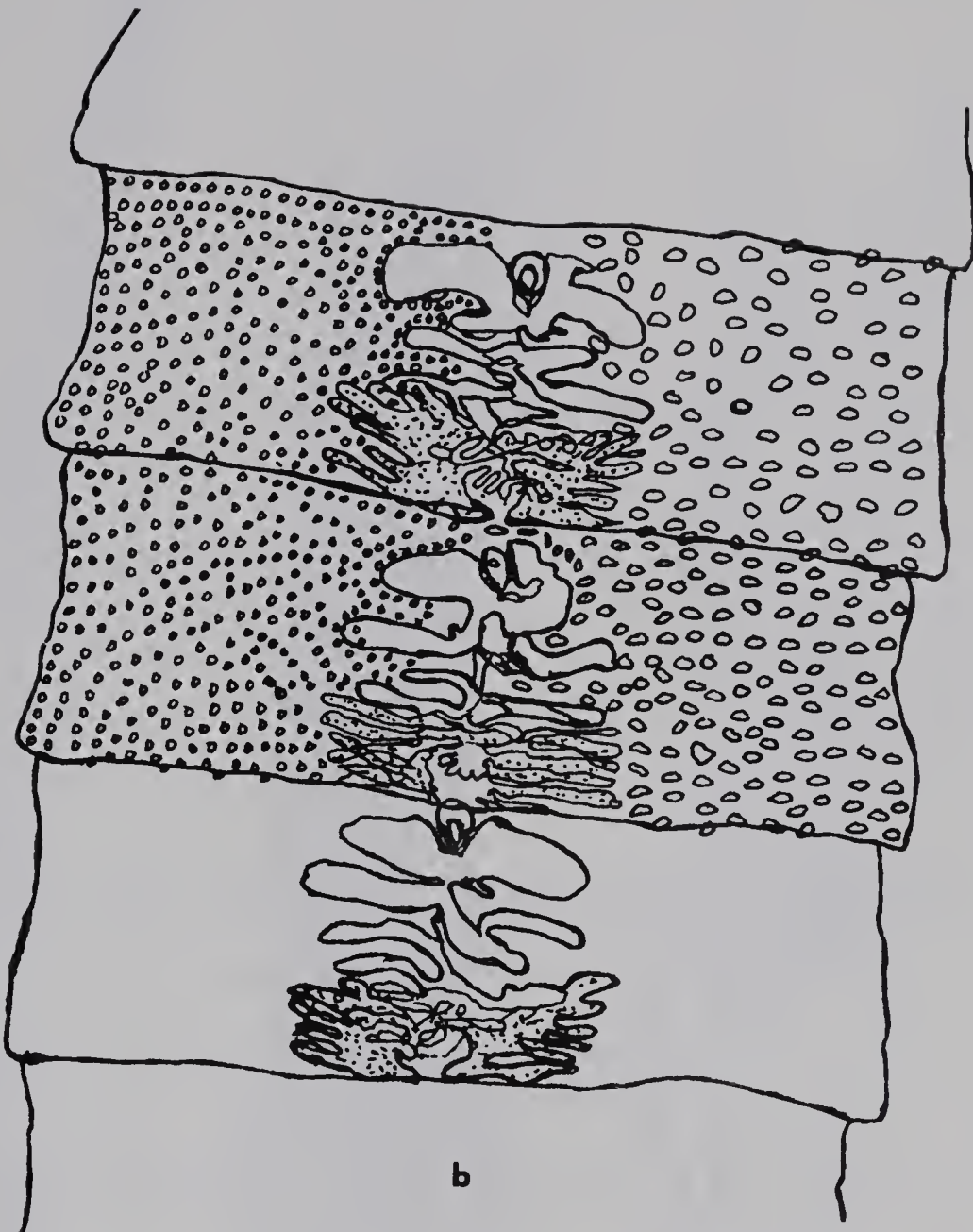
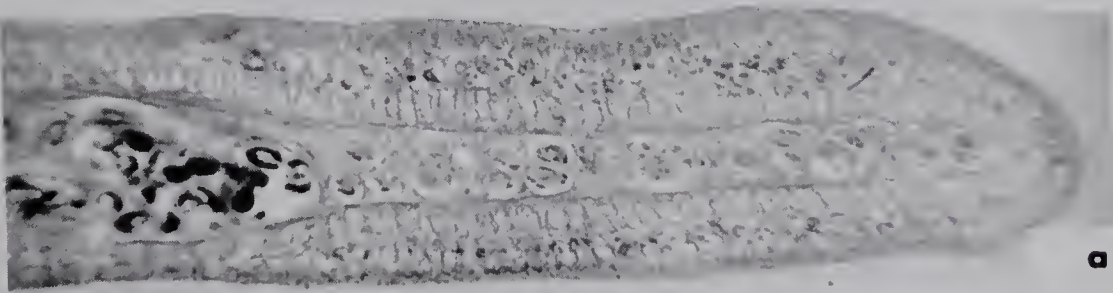


Fig. 18 - D. cordiceps - sections through mature proglottids.

a - transverse section. X12.

b - sagittal sections between midline and lateral nerve cord. X15.

c - sagittal section through midline. X20.



□



□



□



□



□

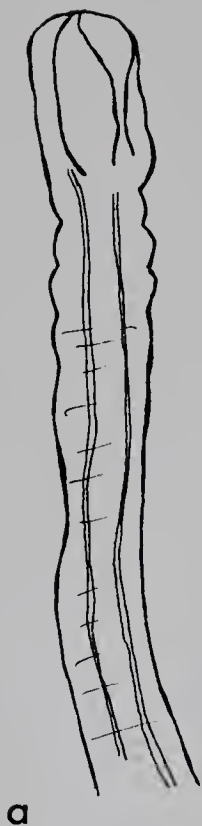
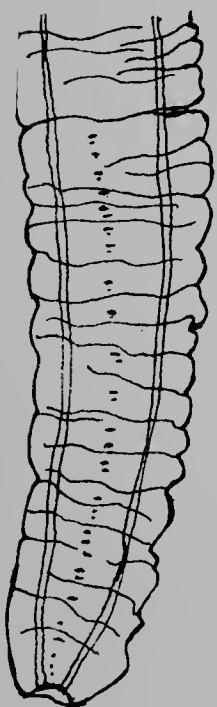
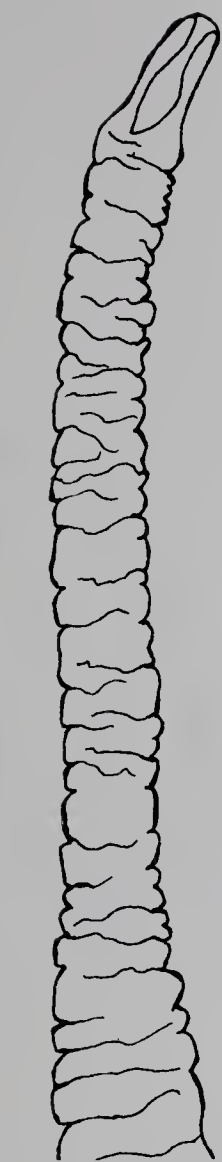
Fig. 19 - The plerocercoid of D. cordiceps.

a - anterior portion of worm. X8.

b - posterior portion of worm. X10.

c - transverse sections through scolex. X35.

d - transverse section through body. X35.



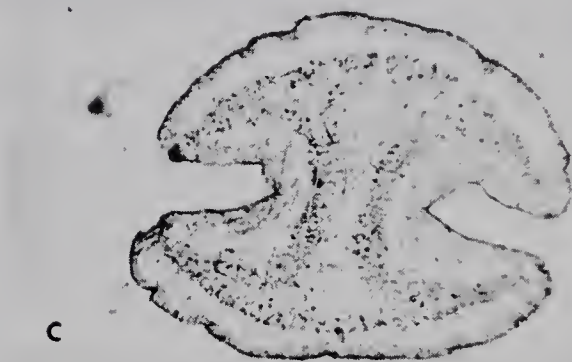
a



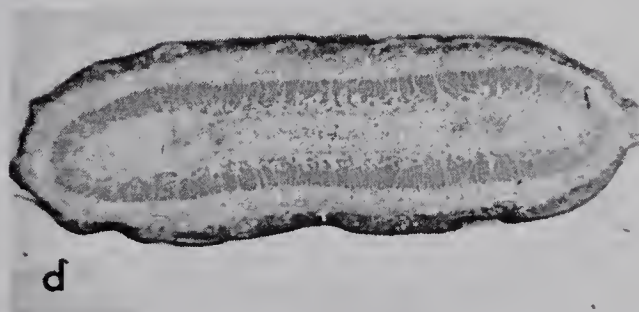
b



c



c



d

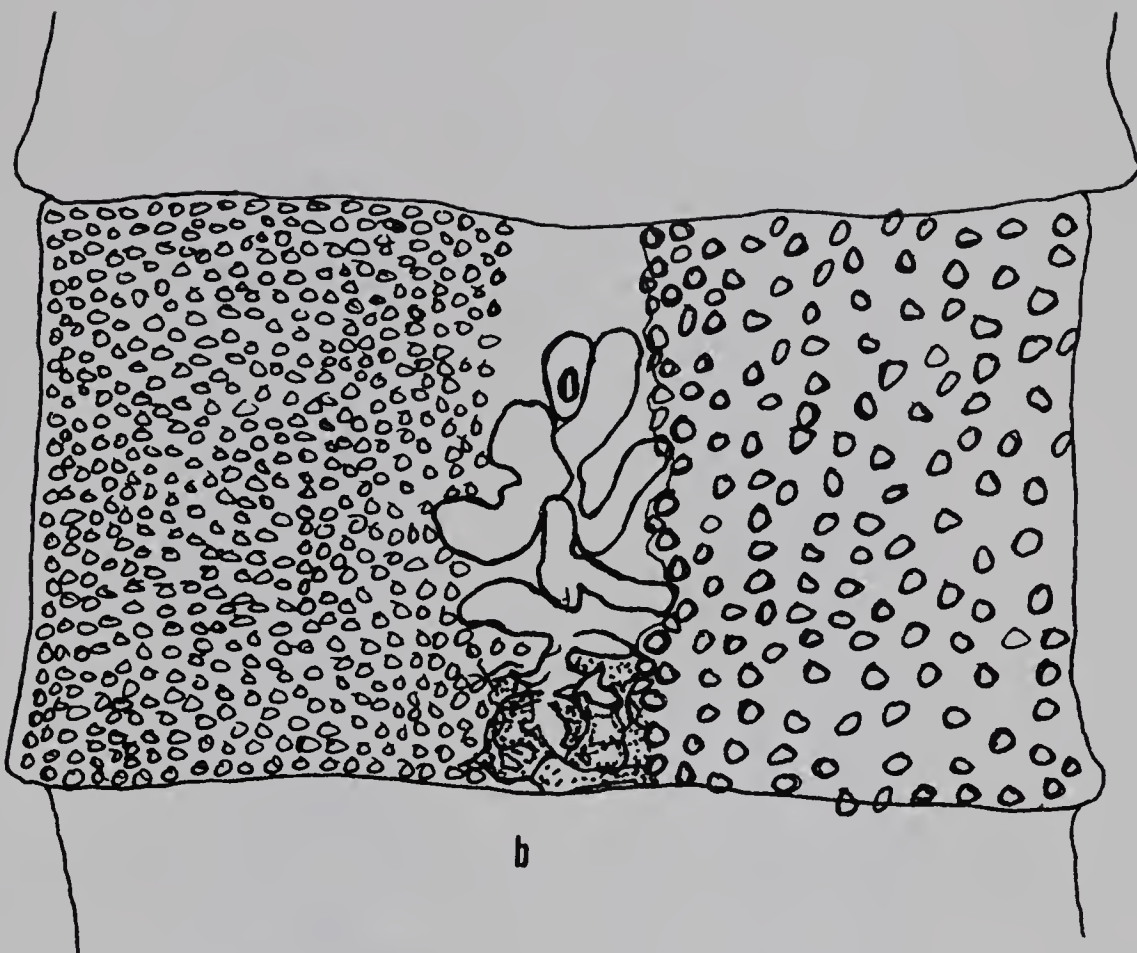
Fig. 20 - D. latum - scolex and proglottids. X12.

a - scolex and anterior portion of worm.

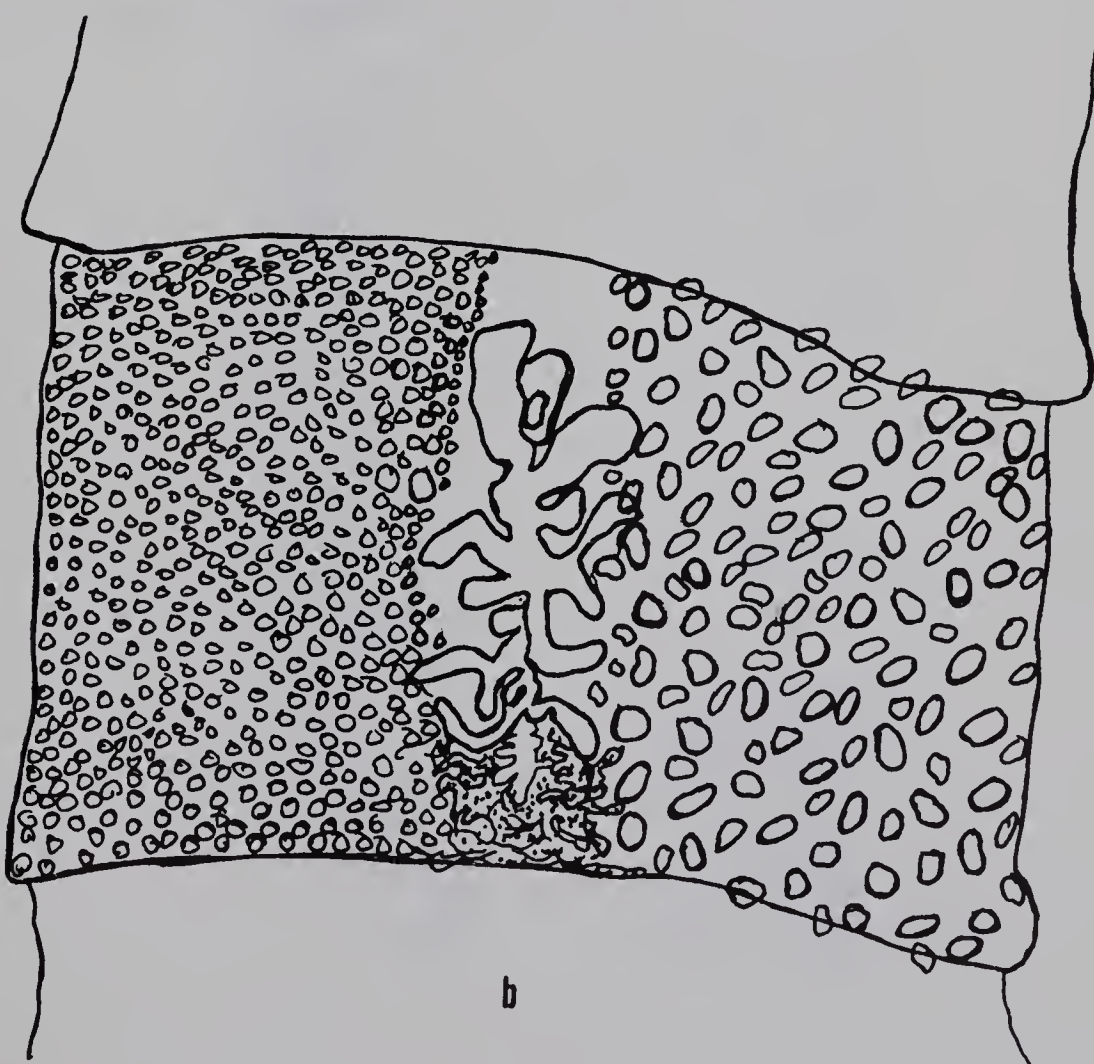
b - fully mature proglottids.



a



b



b

Fig. 21 - D. latum - sections through mature proglottids. X20.

a - portion of transverse section.

b - sagittal section through midline.



□



□

□



Fig. 22 - The plerocercoid of D. latum.

a - complete worms. X10.

b - sections through body. X50.

Fig. 23 - The plerocercoid Diphyllbothrium sp. Type I.

a - complete worms. X10.

b - transverse section through scolex. X50.

c - transverse section through body. X55.

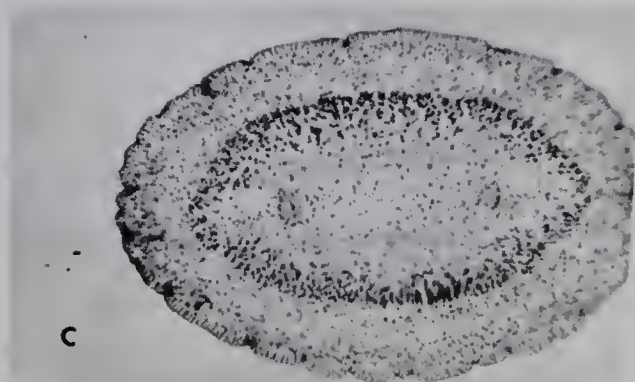
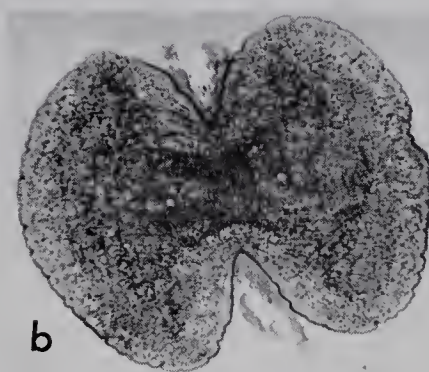
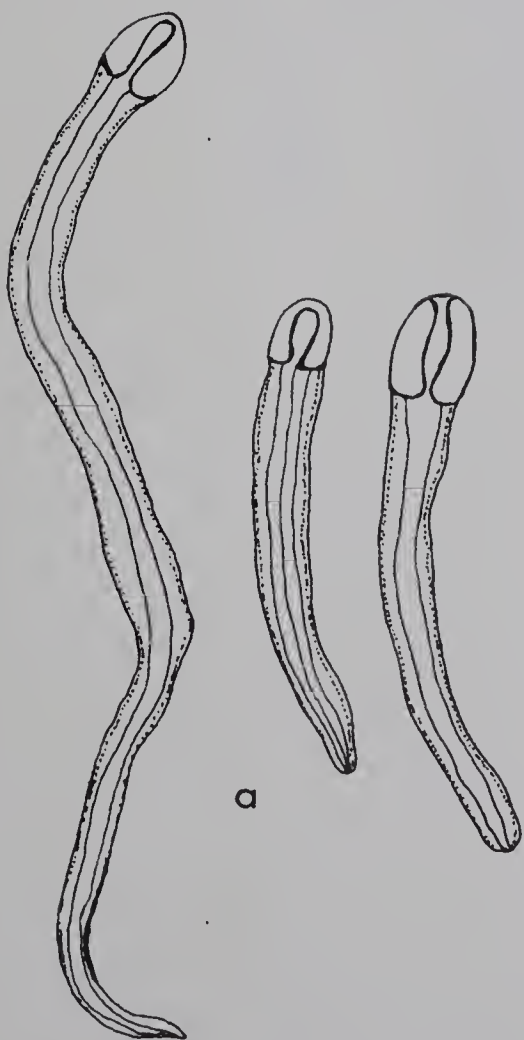
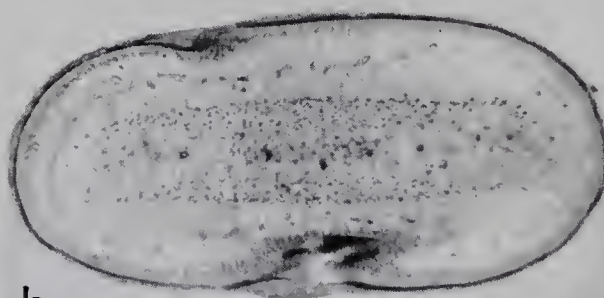
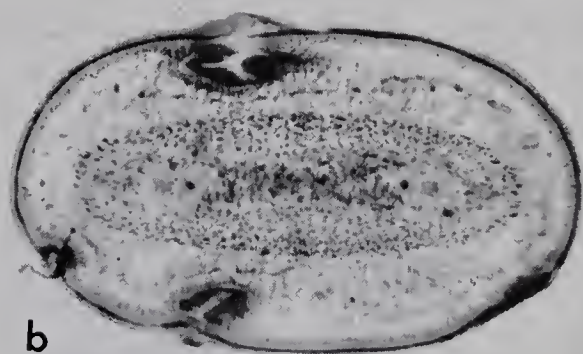
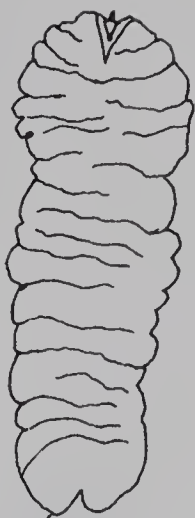
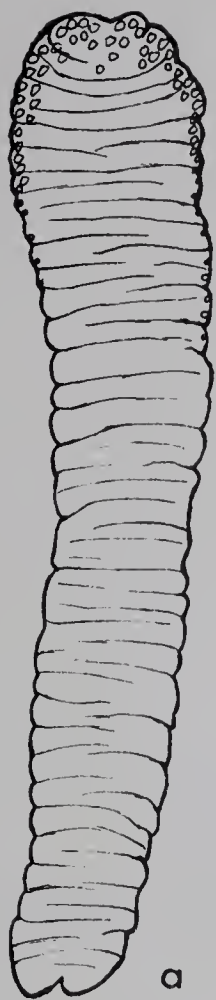


Fig. 24 - The plerocercoid of Diphyllbothrium sp. Type II.

a - complete worms. X8.

b - transverse sections through scolex. X45.

c - transverse sections through body. X60.

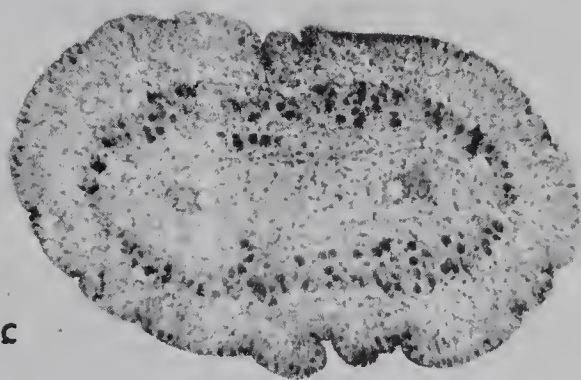
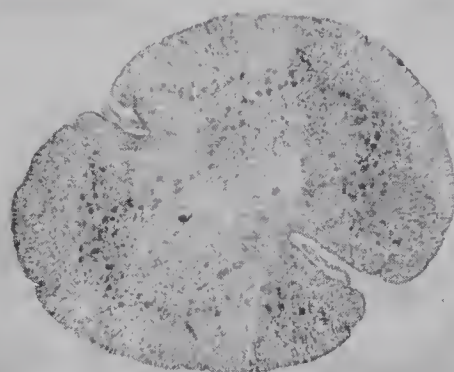
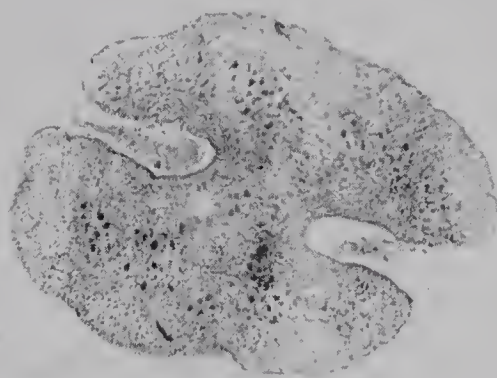
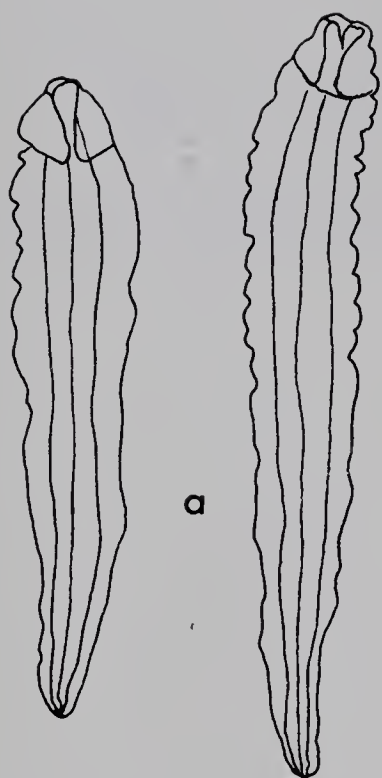
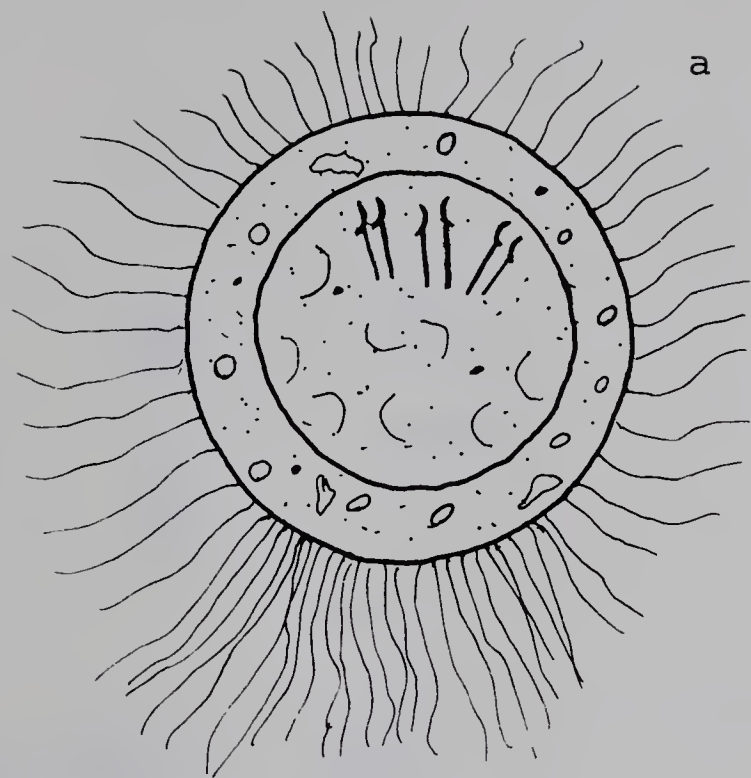


Fig. 25 - The coracidia of three species of Diphyllbothrium. X900.

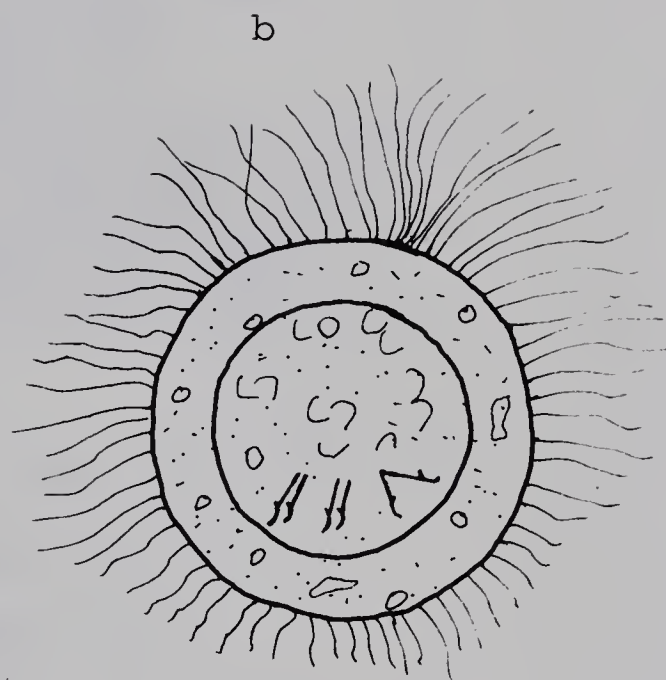
a - D. dendriticum.

b - D. ditremum.

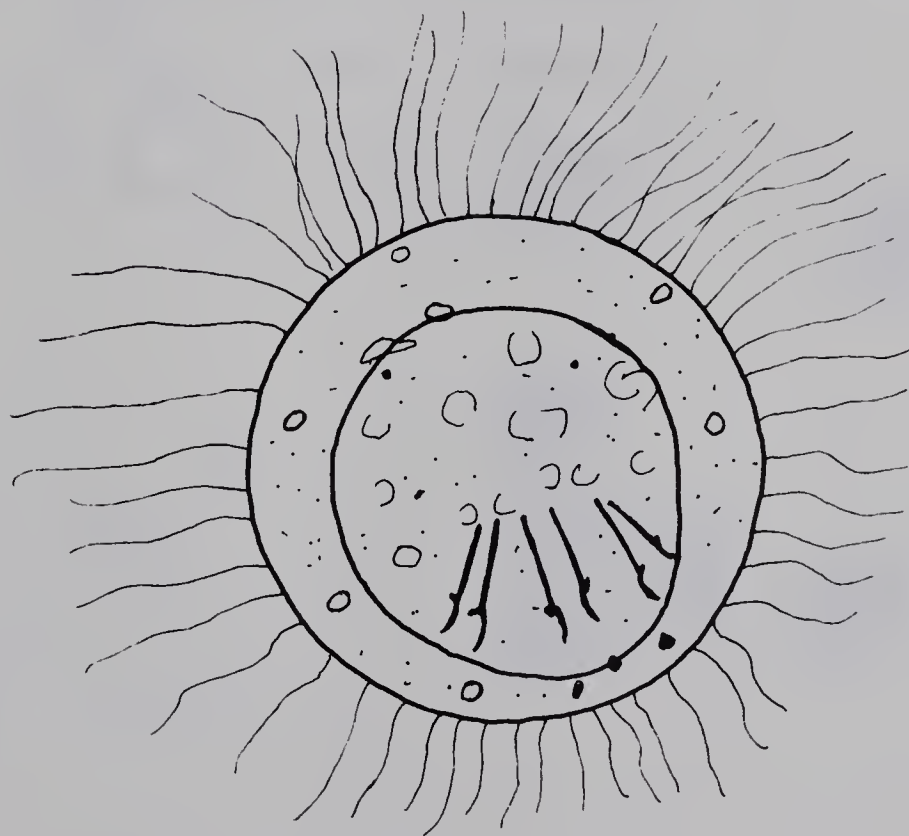
c - D. cordiceps.



a



b



c

Fig. 26 - The eggs of five species of Diphyllbothrium.

a - D. cordiceps. X140.

b - D. dendriticum. X100.

c - D. ditremum. X100.

d - D. osmeri. X105.

e - D. latum. X250.

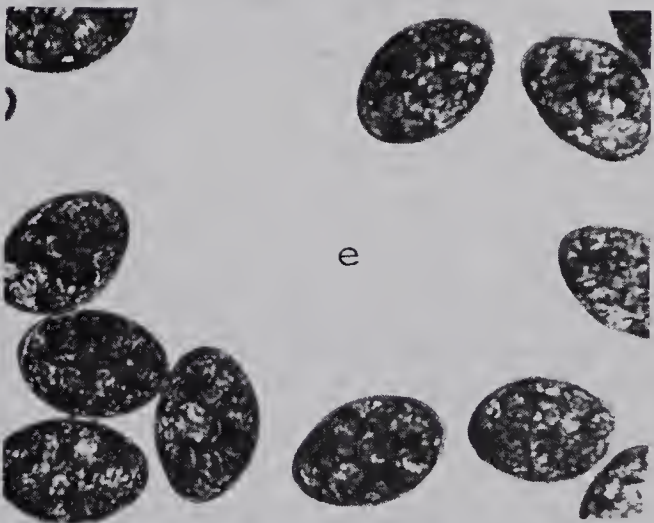
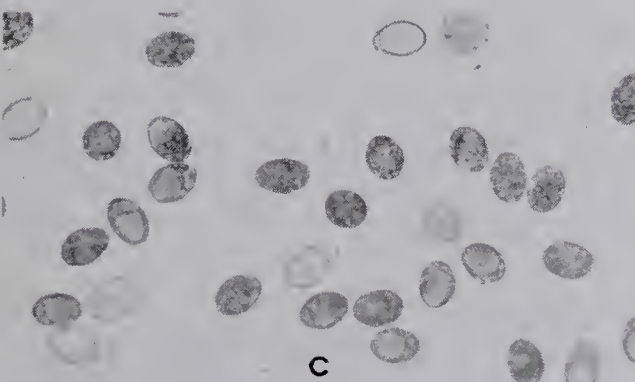
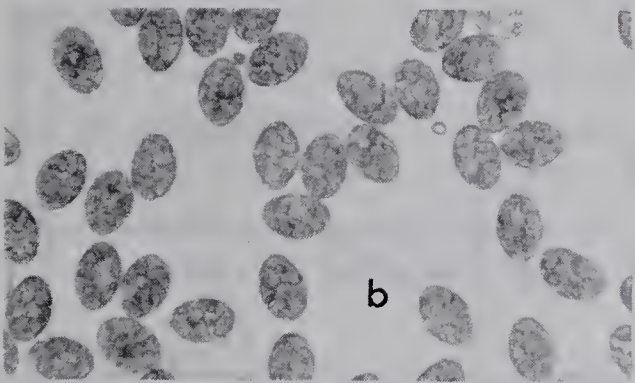
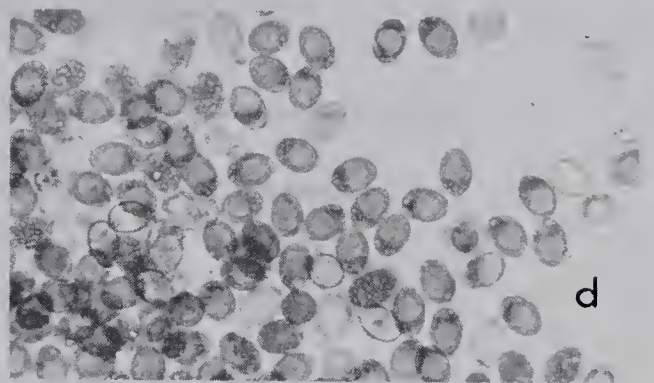


Table 1

Comparative values from three sources for
some characteristics of D. ditremum

Characteristic	Kuhlow (1953b)	Markowski (1949)	Present study
Scolex	1.85 mm.	1.5 mm.	1.6-2.3 mm.
Testes (diameter)	35-65 μ	180x165- 150x275 μ	72x72- 188x160 μ
Cirrus-pouch	160-175x 200-230 μ	270x300 μ	232-362x 188-290 μ
Vitelline gland	28x45 μ	72.6-89x 76-99 μ	51x51- 102x65 μ

Table 2

Record of D. latum infections in man and dog in Alberta and the Northwest Territories, 1949-1965*

Location	Host	Ethnic origin	No. of records
Brocket, Alberta	Man	Indian	1
Gleichen, Alberta	"	"	1
Desmarais, Alberta	"	"	1
Chard, Alberta	"	"	1
Wabasco, Alberta	"	"	1
Fort Chipewyan, Alta.	"	"	?
Mayerthorpe, Alta.	"	European	2
Other Alberta records (locations unknown)	"	"	5
	"	Indian	3
Cold Lake, Alberta	Dog		2
Lac La Biche, Alta.	"		1
Wabamun Lake, Alta.	"		1
Fort Providence, N.W.T.	Man	Indian	3
Fort Simpson, N.W.T.	"	"	2
Fort Rae, N.W.T.	"	"	1
Martin Lake, N.W.T.	"	"	2
Reid Island, N.W.T.	"	Eskimo	1
Coppermine, N.W.T.	"	"	2
Spence Bay, N.W.T.	"	"	1
Gjoa Haven, N.W.T.	"	"	1
Aklavik, N.W.T.	"	"	1
Lac La Martre, N.W.T.	"	Indian	1
Holman Island, N.W.T.	"	Eskimo	1
Pelly Bay, N.W.T.	"	"	1
Tuktoyaktuk, N.W.T.	"	"	1

*Compiled from the records of the Provincial Laboratory of Public Health and the Charles Camshell Hospital, Edmonton, and from material identified by the author.

In addition, D. latum plerocercoids have been found in pike and walleye from Cold Lake, Lac La Biche and Rock Island Lake, Alberta, and once in whitefish from Lac Ste. Anne, Alberta.

Table 3

Summary of some adult characteristics of the five species of Diphylllobothrium

Characteristic	<u>D. dendriticum</u>	<u>D. ditremum</u>	<u>D. osmeri</u>	<u>D. cordiceps</u>	<u>D. latum</u>
Scolex	lanceolate 2.0-2.5 (1.5-1.8 mm in birds)	cordate, ovate-cordate, cordate-lanceolate. 1.6-2.3 by 0.9-1.3 mm.	spatulate, lanceolate 1.8-2.5 by .7-.8 mm.	ovate, lanceolate. 2.0-2.3x.75-.9 mm.	spatulate, some- times lanceolate. 2.0-3.2x1.0-1.2 mm.
Neck	long	very short	short	long	long
Strobila size (relative) gestalt	large thick, serrated, spindle-shaped	medium-sized thick, serrated, spindle-shaped	small flat, attenuated	large rugose, moderately serrated	large ribbon-like, uniform width
Proglottids shape	trapezoidal, quadrate, oblong 4:1 to 1:3	trapezoidal, quadrate, oblong 3:1 to 1:3	trapezoidal to oblong 2:1 to 4:7	trapezoidal, rectangular oblong up to 7:1	rectangular
width-length ratio					2:1
Testes size	102x87 to 188x102 μ	72x72 - 188x160 μ	72.5-130x72.5-116 μ	116-276x102-246 μ	131x131 to 218x138 μ
shape	ovoidal but variable	spherical but variable	ovoidal to reniform	ovoidal but variable	ovoidal, pyriform, spherical
Vitellaria size	36x36-72x51 μ	51x51 to 102x65 μ	44x44 to 72x58 μ	51x44 to 72x58 μ	58x44 to 131-87 μ
shape	mainly ovoidal but variable	spherical, pyriform	mainly spherical or ovoidal	spherical or ovoidal but variable	spherical, ovoidal
Uterus width-length ratio	1:1	3:5	1:2	6:5	1:2
number of loops	usually 5-8 pairs	usually 4-5 pairs	usually 9-12 pairs	6-7 pairs	6-10 pairs
Cirrus pouch size	290-420x160-290 μ	232-362x188-290 μ	246-435x174-290 μ	290-508x188-450 μ	580-609x406-435 μ
shape	ovoidal	spherical	ovoidal	very variable - ovate to top-shaped	pyriform
Muscle fibres (longitudinal) development and arrangement (seen in t.s.)	well-developed; in bundles in deep layer	poorly developed; as individual units	poorly developed; as individual units	well-developed; in bundles in deep layer	well-developed, in distinct bundles

Summary of some characteristics of the seven species of *Diphylllobothrium plerocercoids*

Characteristic	<u>D. dendriticum</u>	<u>D. ditremum</u>	<u>D. osmeri</u>	<u>D. cordiceps</u>	<u>D. latum</u>	<u>D. sp.I</u>	<u>D. sp.II</u>
Size	large 1.0 - 30 cm.	small to medium sized; less than 2 mm.-14 mm.	medium-sized; 8.17 mm.	large 1.0-15 cm.	large 4-50 cm.	small to medium-sized, 3.0 - 12 mm.	medium-sized; 8 - 12 mm.
Shape	thick, uniform width; posterior invagination	dorso-ventrally flattened; prominently shouldered	long, slender, cylindrical or somewhat rectangular	dorso-ventrally flattened	dorso-ventrally flattened; marked post invagination	attenuated posteriorly	tapers posteriorly; dorso-ventrally flattened
Cuticle	deeply wrinkled; bristle-covered	moderately wrinkled; bristle-covered; 11 - 14.5 μ thick	smooth; bristle- covered; 5.5 - 9.0 μ thick	moderately wrinkled; 25 - 30 μ thick	deeply wrinkled; without bristles 11-14 μ thick	smooth; bristle-covered 5 - 11 μ thick	moderately wrinkled; bristle-covered 4 - 5.5 μ thick
Cuticular bristles	very short, 3 - 5 μ	long, - 29 μ 14.5	11 - 14.5 μ	none	none	5 - 11 μ	11 - 18 μ
Scolex	laterally compressed; ovate; evaginated	laterally compressed; acuminate; evaginated	lanceolate; evaginated	ovate to ovate-lanceolate, evaginated	invaginated	olivolate or roundish; evaginated	not well delimited from body
Frontal glands	poorly developed; extend in ant. portion of body	well-developed; extend in ant. portion of body	well-developed; confined to scolex	poorly developed; confined to scolex	well-developed; extend well in body	poorly developed; confined to scolex	poorly developed; as single cells or clumps of few cells in scolex only
Longitudinal muscle layer	well-developed; 130 - 160 μ thick not in bundles	well-developed; variable thickness as single fibres or loose bundles	well-developed; in bundles	well-developed; about 90 μ thick; in bundles	well-developed; not in bundles	well-developed; about 60 μ thick	well-developed; about 36 μ thick

V - STUDIES ON THE RATE AND PATTERN OF GROWTH

The rate and pattern of growth of D. dendriticum, D. ditremum, D. osmeri, and D. cordiceps, raised in different species of hosts, were investigated. The mean length of the worms recovered at the various intervals at which the hosts were sacrificed or dewormed, was used as the measure of growth. Dry weight, though more accurate as a measure of growth in cestodes, could not be used in these studies since this procedure destroys the worms, which, in the present case, were required for histological and other studies.

Data for D. dendriticum reared in dogs, cats, rats, and gulls, are presented in Table 5. Data for D. ditremum, reared in the same four host species are shown in Table 6. Those for D. osmeri are recorded in Table 7, and those for D. cordiceps, which were reared only in dogs and cats, in Table 8. The plerocercoids of D. dendriticum used were all 3 to 4 cm. long; those of D. ditremum were 6 to 10 mm; those of D. osmeri, 12 to 16 mm; and those of D. cordiceps, 5 to 7 cm. in length.

Such considerable variations in strobilar length occurred among fully relaxed specimens of a single species even raised simultaneously in the same host, or under identical conditions in hosts of the same species, that no reliable rate of growth could be determined for any of the species. For example, the eleven, 10-day old specimens of D. dendriticum reared in a single dog, and

which showed no evidence of apolysis, ranged in length from 7.0 cm. to 200 cm. - seven of them were between 109 and 200 cm. long, and the others were between 7 and 17.5 cm. in length.

Although, on the basis of the data obtained, a reliable rate of growth could not be established, when the mean length of the worms obtained from each host species at each interval is calculated and plotted, a basic triphasic pattern is recognized: an initial period of rapid growth during which the worms become fully gravid, and in some cases attain maximum length as well, is followed by a period of slower growth or maintenance of size, and then by a final period of decrease in size. Figs. 27 and 28 are growth curves for D. dendriticum and D. ditremum based on the logarithmic values (\log_{10}) of the mean lengths.

The duration of the first phase roughly corresponds to the duration of the prepatent period, which was 6-8 days in all species except for D. latum, in which it was 20-28 days for specimens from dogs and even longer for those from cats, and D. ditremum from cats, in which the prepatent period was usually 9-12 days.

The prepatent period observed for D. latum in this study falls within the 14-32 day range reported by other workers (summarized by Kuhlowl, 1955). The author's observations agree with those of Kuhlowl (1953c) on D. dendriticum but not on D. osmeri for which he states (p. 209), "...dass die Eier von

Diphyllbothrium osmeri, wenn überhaupt, nicht eher als etwa 30 Tage nach der Fütterung auftraten". The literature contains no comparable data on D. cordiceps, and the only information on D. ditremum is given by Kuhlow (1953b) who found eggs in the stool on the 15th day after infection. His observations, however, could not provide information on precisely when eggs first appeared in the feces.

The length of the second phase seems to be a function of the mean relative rates of growth and apolysis. Apolysis occurred earlier in species with large strobilae than in those with small ones, and at different times for the same species raised in different species of hosts. Some specimens of D. cordiceps raised in dogs and cats were apolyzed by the 10th day; D. dendriticum from dogs and rats between the 15th and 20th day, and from gulls and cats by the 10th day; but D. ditremum and D. osmeri usually did not show any evidence of apolysis until around the 30th day. Such variations in the onset of apolysis will therefore definitely influence the length of the second phase.

The third phase is obviously the net product of a greater rate of apolysis than of growth, and consequently, determines the longevity of the worms in the different species of hosts. All species studied lived longer in dogs than in any other host, and had their shortest life-span in gulls. Relatively old strobilae usually had an emaciated appearance, and contained

many empty uteri or uteri which contained only unripe eggs in their proximal loops, and a few ripe ones in other loops. Some uteri were broken into two or more tandem parts, thus presenting the appearance of two or more serially arranged uteri per proglottid; and in some cases, the centrally located components of the genital complex were competely removed from proglottids of the terminal third of the strobila.

It can be seen that even though the pattern of growth was the same in all hosts, the rate of growth was different, being most rapid in dogs, in which all species attained their maximum size. The type of diet was probably a contributory factor, but size of intestinal lumen definitely seems to be important in this pattern, which is very similar to that of Hymenolepis nana reared in the grey squirrel (Schiller, 1959). In his comparative study of the rate and pattern of growth of this cestode in three different host species, Schiller noted a faster growth rate in grey squirrels, in which the worms reached maximum size and matured earliest.

Because different investigators have used different criteria to define growth, direct and accurate comparison with some studies obviously cannot be made. In other cases, however, despite the difference in criteria, some basic similarities in pattern of growth can be discerned. Thus, basically the same pattern discussed above has been reported for species of Diphyllbothrium

by Archer and Hopkins (1958) and by Bråten (1966), except that the initial period of lag reported by them was not observed in the present study.

From these observations and from other examples in the literature, it would seem that this pattern is basic to the growth of tapeworms in general. Some data on the growth and maturation of D. dendriticum, D. ditremum, D. osmeri, and D. cordiceps are recorded in Table 12.

There is abundant evidence in the literature that high population densities may result in diminution of size, retardation of maturation, or inhibition of reproduction in cestodes (Weinmann, 1958; Read, 1959; Holmes, 1961; Roberts, 1961). Reduction in size in heavy infections with Diphyllbothrium has been observed by Petrushevski and Tarasov (1933) in D. latum from man; Wardle and Green (1941) in the same species from dogs; and Kuhlow (1953c) in D. dendriticum from gulls. Rausch (1954) suggested that "crowding undoubtedly exerts a retarding influence on strobilar growth".

In the present study, the effects of high intensity of infection on D. dendriticum and D. ditremum, especially upon their size, were investigated in rats, pups, and kittens. If 20 or more plerocercoids of D. dendriticum were fed to rats, none got established. On two occasions, autopsy 24 hours post-infection yielded three partially digested worms from the stomachs of two hosts, and what appeared to be remains of worms in their ileum. On other occasions, no trace of the worms was found despite

thorough examination of the hosts. From Table 9 it is seen that as the number of plerocercoids per feeding decreased, the percentage "take" increased, reaching a maximum percentage when feedings of 4-5 plerocercoids were used. As time passed, and the worms increased in size, the number recovered gradually decreased. When large numbers of this species of plerocercoid (50-100 worms) were fed to pups and kittens only a few became established and reached maturity. Their size was essentially the same as those in control infections (Table 10). As in rats, the worm burden proportionately decreased as the size of the worms increased.

In D. ditremum, the situation was roughly analogous to that of most of the Cyclophyllideans which have been studied. Most of the worms became established, heavy infections were maintained, and a reduction in mean size occurred (Table 11). Maturation was not affected, and although a decrease in the density of infection with time occurred it was not as marked as in D. dendriticum. In heavy infections in which 100-200 plerocercoids of D. ditremum were fed to pups, an extension of the range beyond the preferred site, namely, the mid-portion of the jejunum, into the ileum, occurred. The mean sizes of specimens from these two locations were significantly different (Table 11a). This size difference may not, however, be attributable to crowding per se, but rather to environmental factors, specifically to the difference in quantity and nature of nutrients,

present.

There are some interesting parallels in the literature to these two reactions. Similar to the reaction of D. dendriticum in which no extension of the intra-intestinal range occurred, are the reactions of D. latum reported by Petrushevski and Tarasov and by Wardle and Green (supra cit.), and that reported by Holmes (1961) for the Acanthocephala, Moniliiformis dubius. In all these cases, reduction in the number of worms, and maintenance of size occurred. Contrary to this reaction is that observed for D. ditremum in the present study, for Ancylostoma caninum (Krupp, 1961), for Hymenolepis diminuta (Holmes, 1961), and for some other helminths, in which case extension of the intra-intestinal range, diminution of size, and only slight reductions in numbers occurred.

These differences between the reactions of D. dendriticum and D. ditremum may however be two approaches to the same basic objective, for in both cases, a compensatory mechanism seems to regulate total biomass of worms in accordance with the carrying capacity of the intestine as determined by the available space and nutrients. In one case, this is accomplished by a reduction in the number of worms and a greater realization of size potential; in the other case, a high intensity is maintained, but the size of the worms is reduced. Further study of these reactions may furnish some useful information on the intra-intestinal ecology of helminths.

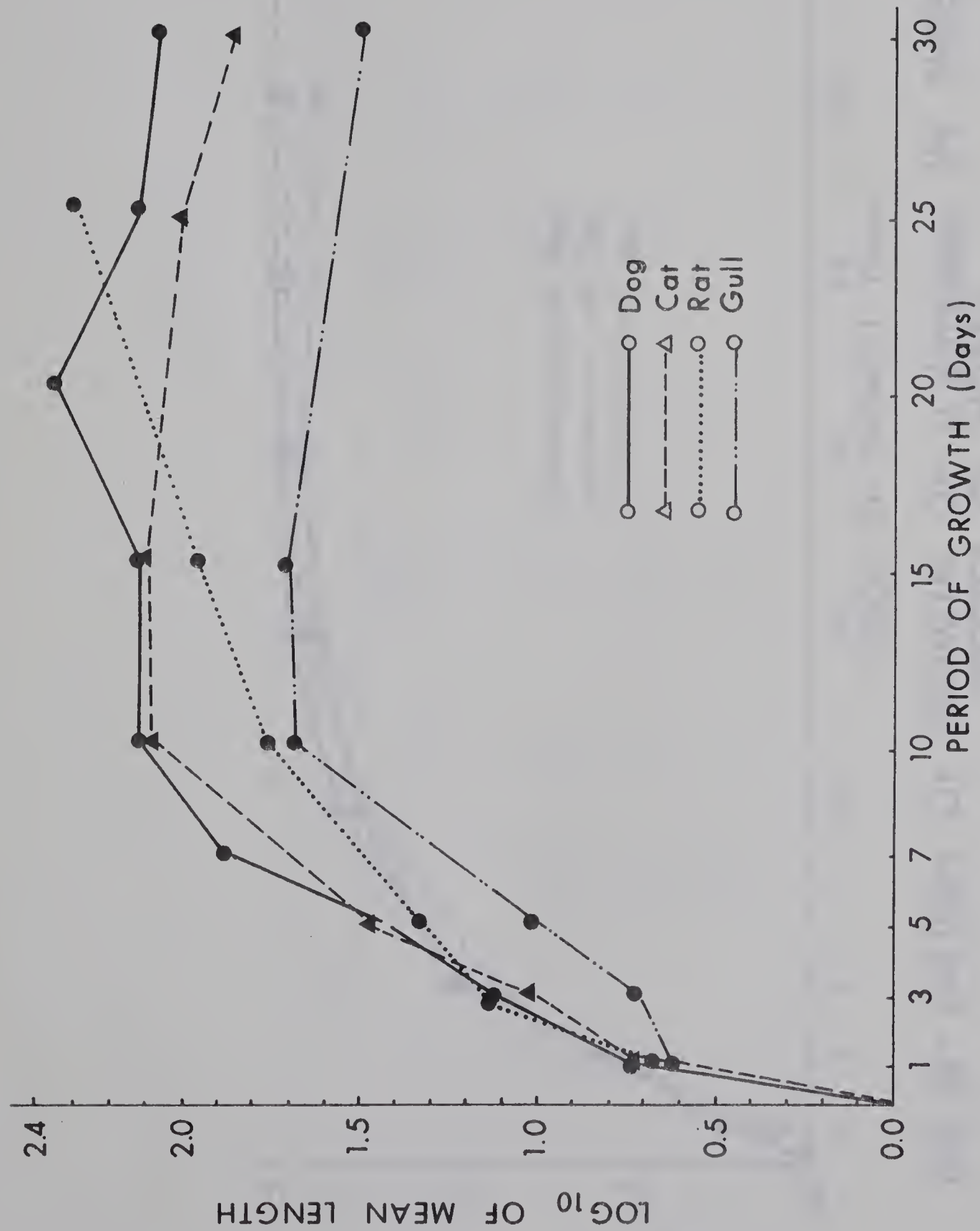


FIG. 27—PATTERN OF GROWTH OF *D. dendriticum* IN
FOUR SPECIES OF HOSTS

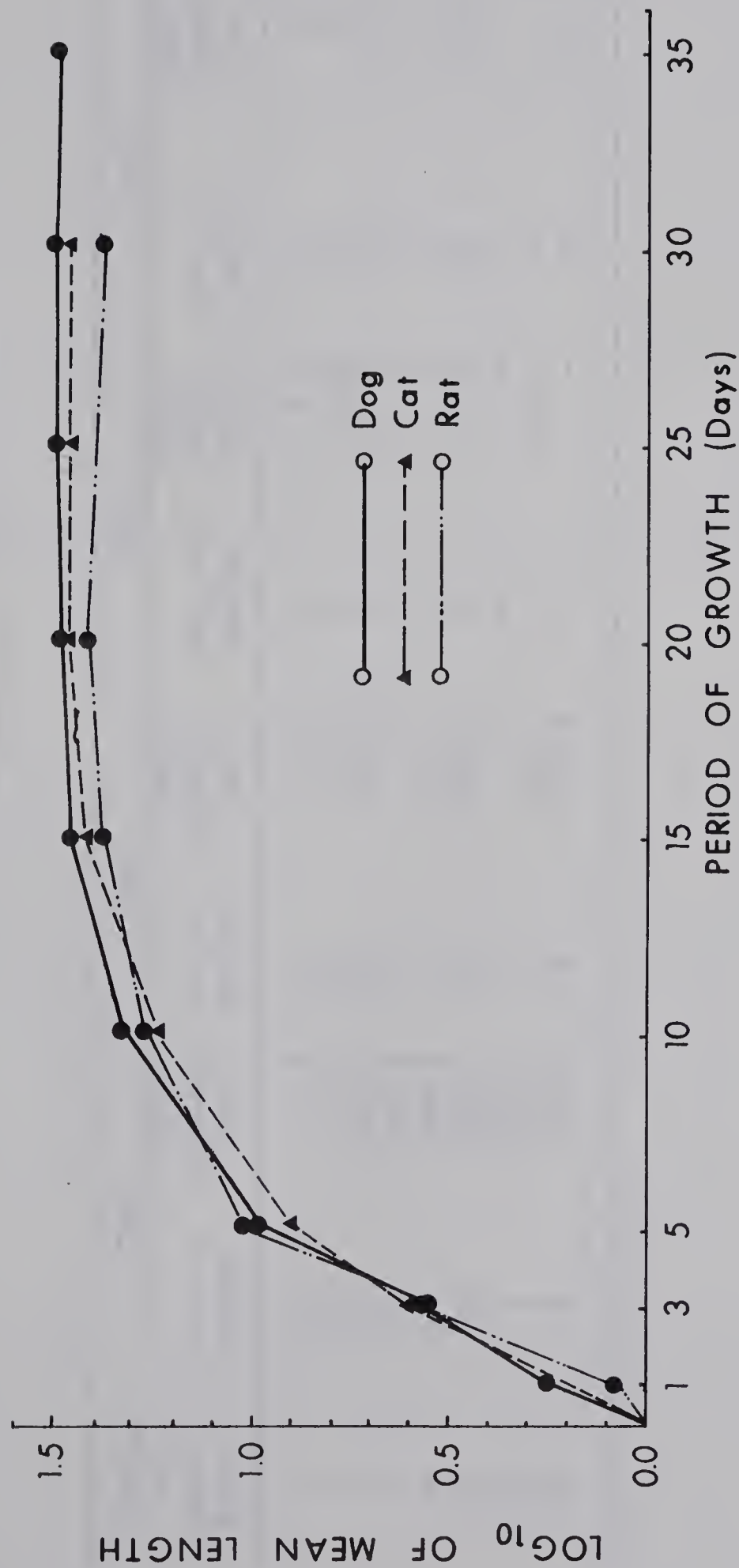


FIG. 28 - PATTERN OF GROWTH OF *D. ditremum* IN THREE SPECIES OF HOSTS

Table 5
Data on the rate of growth of D. dendriticum
(10-20 plerocercoids fed)

Period of Growth days	Dog		Cat		Rat		Gull	
	No. of worms	Mean Length (cm.)	No. of worms	Mean Length (cm.)	No. of worms	Mean Length (cm.)	No. of worms	Mean Length (cm.)
1	20	5.4	17	5.3	14	4.8	20	4.3
3	19	13.1	20	10.8	9	13.2	37	5.2
5	39	27.0	25	29.6	4	21.2	23	10.3
7	11	76.2	-	-	-	-	-	-
10	26	128.8	8	121.5	9	57.5	16	48.5
15	18	130.2	16	130.0	3	91.5	12	51.5
20	2	228.0	-	-	-	-	-	-
25	8	131.6	2	100.7	1	163.0	-	-
30	6	117.5	2	72.4	-	-	4	31.6

Table 6

Data on the rate of growth of D. ditremum

Period of Growth (dys.)	Dog		Cat		Rat		Gull	
	No.of worms	Mean Length	No.of worms	Mean Length	No.of worms	Mean Length	No.of worms	Mean Length
1	44	1.8	26	1.6	16	1.6	13	1.2
3	49	3.8	4	4.2	-	-	8	3.6
5	80	9.5	19	8.2	20	7.6	23	10.4
7	-	-	-	-	-	-	-	-
10	67	21.6	22	17.6	11	19.6	16	18.3
15	92	29.8	26	26.6	15	21.9	12	24.5
20	22	31.0	11	30.2			7	26.2
25	16	31.4	5	29.0				-
30	44	31.9	14	29.4			8	24.0
35	11	30.7	-	-				-

Table 7

Data on the rate of growth of D. osmeri

Period of Growth (dys.)	Dog		Rat		Gull*		Cat*	
	No.of worms	Mean Length	No.of worms	Mean Length	No.of worms	Mean Length	No.of worms	Mean Length
1	18	1.5	10	1.6	24	1.2	10	1.0
5	15	2.8	4	7.4	8	5.7	10	3.0
10	9	10.6	3	19.8	8	9.6	13	2.2
15	3	30.3	5	26.3	4	18.0	17	12.2
20	-	-	-	-	9	25.5	-	-
25	-	-	-	-	-	-	-	-
30	3	30.9	1	30.6	2	24.4	-	-
35	4	30.2	1	30.6	3	24.0	-	-

*Mainly immature worms

Table 8

Data on the rate of growth of D. cordiceps

Period of Growth (dys.)	Dog		Cat	
	No.of worms	Mean Length (cm.)	No.of worms	Mean Length (cm.)
1	3	8.1	5	7.8
5	5	35.3	5	33.6
10	3	256.5	2	199.8
15	4	268.3	2	239.3
20	1	211.4	1	166.2
25	1	207.5	-	150.7
30	2	210.3	1	143.2

Table 9

Relationship between number of D. dendriticum
plerocercoids fed to rats and
the % "take" by the 5th day

No.of pleros. fed	No.of worms establ.	% of estab.	Mean Length (cm.)
25	0	0	0
20	0	0	0
15	7	46.7	21.0
10	8	80.0	20.5
7	6	85.7	20.7
5	5	100	21.2
4	4	100	21.0
3	3	100	20.9

Table 10

Size of D. dendriticum in heavy infections
(50-100 plerocercoids fed)

Period of Growth (dys.)	Dog		Cat	
	No.of worms	Mean Length (cm.)	No.of worms	Mean Length (cm.)
1	48	5.5	43	5.5
3	19	12.8	22	9.8
5	17	27.7	22	27.9
10	6	131.6	3	124.0
15	6	130.2	3	128.1
20	4	220.9	-	-
25	10	128.8	-	-
30	4	116.0	1	119.8

Table 11

Size of D. ditremum in heavy infections
(100-200 plerocercoids fed)

Period of Growth (dys.)	Dog		Cat	
	No.of worms	Mean Length (cm.)	No.of worms	Mean Length (cm.)
1	180	1.2	93	1.4
5	169	9.9	89	8.0
10	160	16.0	77	14.2
15	167	19.7	63	19.0
20	152	22.2	60	21.8
25	-	-	-	-
30	148	22.0	53	22.0

Table 11A

Mean lengths of D. ditremum from the
jejunum and the ileum of pups (heavy infections)

Period of Growth (dys.)	Jejunum		Ileum	
	No.of worms	Mean Length (cm.)	No.of worms	Mean Length (cm.)
5	106	10.3	63	9.4
10	120	18.2	40	16.1
15	133	23.9	34	15.6
20	100	26.8	52	18.6
25	-	-	-	-
30	108	26.0	40	18.1

ECOLOGY

Examination of Fish

Of the 14 species of fish examined, only 5 species belonging to 3 of the 9 families represented, were infected with Diphyllbothrium plerocercoids (Table 13). The infected species included three salmonids from Kootenay and Trout Lakes, namely, kokanee, rainbow trout, and dolly varden char; and northern pike and walleye from Iosegun Lake.

The overall extensity and intensity of infection were different for each of the five infected fish species. Practically 100% of the kokanee were infected with 5 different species of plerocercoids, but only 13.8% of the 65 pike were found infected with but a single species. The mean intensity of infection in rainbow trout was 59.8 plerocercoids of 7 different species; it was less than two plerocercoids in pike.

The species composition of the plerocercoids obtained from these five host species during the 1965-1966 period is shown in Table 14. During this period, D. ditremum was the most abundant species in the salmonids, accounting for about three-fourths of all plerocercoids in these fish. D. osmeri and D. dendriticum were moderately abundant (the latter especially in trout), but the other four species were not common in salmonids. In particular, D. latum, which was the only species found in pike and walleye, was virtually unrepresented in the salmonids. It was found only

twice in Kootenay Lake trout: in August 1964, and in June 1965.

Table 15 shows the total percentage of specimens (1963-1966) infected in different organs with one or more of the seven species of plerocercoids; in Table 16, these percentages are expressed in terms of the number of infected fish of each species.

Only 1% of the total number of plerocercoids was located in the musculature, and more worms were unencysted than encysted (Table 17). In kokanee and dolly varden, a much higher percentage was encysted than unencysted; in trout, these percentages were about equal; and in pike and walleye, the worms were nearly all unencysted.

The frequency of infection with each species in different organs of the 476 kokanee and 34 rainbow trout found infected during 1965-1966 is presented in Table 18. Except for D. cordiceps, which was found more often in the musculature of trout than in any other site, all species were most frequently found in locations on the digestive tract (serosa of the stomach, the caeca and the perigastrial fat). All infected kokanee and trout carried plerocercoids of D. ditremum in such locations. Plerocercoids of D. dendriticum were also frequently found in similar sites and others, in trout, but were extremely rare in kokanee. Similarly, D. cordiceps showed a relatively high frequency of occurrence in trout, but did not occur in kokanee. The three plerocercoids of D. latum from trout were lying unencysted in the body cavity.

From the 1963 and 1964 samples of kokanee and rainbow trout, no difference in extensity and/or intensity of infection was evident

in fish of the same size and age classes obtained from six of the eight zones (Fig. 3) into which Kootenay Lake had been divided (Tables 19 and 20). Zoning of the lake for the purpose of more detailed sampling was therefore discontinued after 1964.

Size of host and infection

The intensity but not the extensity of infection in kokanee varied with the size of the host (Table 21). A marked difference existed between the mean intensity in the smallest size class (5.9 plerocercoids per fish) and those of the other classes between which only slight variations occurred. From the data for 1965-1966, compiled in Table 22, this basic pattern is evident for both of the common species, D. ditremum and D. osmeri, in this host.

Data for rainbow trout (Table 23) revealed a positive correlation between size of host and the overall extensity and intensity of infection. Extensity varied from zero among specimens up to 42 cm. (16.5 in.) in length, to 100% in the higher size categories, with a mean intensity of 83.2 worms per infected fish in the latter. Similar relationships are shown in Table 24 for D. dendriticum, D. ditremum, D. cordiceps, and D. osmeri infections in this host during the 1965-1966 period.

Data for dolly varden char are entered in Table 25; those for walleye in Table 26; and those for northern pike, in Table 27. In each case, the data suggest the same relationship between size and infection as observed for rainbow trout.

Age of host and infection

As seen in Table 28 there was virtually no difference in the overall extensity of infection among the three age groups of kokanee. Intensity of infection, however, varied considerably, ranging from a mean of 7.8 in one-year olds, to a mean of 23 in 3-year old specimens. Sixty-six plerocercoids - all from the viscera - were obtained from one three-year old spawning kokanee. It was not unusual to find infections of 40 to 50 worms in these fish. In some cases, most of the serosal surface of the stomach was covered with encysted larvae as illustrated in Fig. 29.

The data in Table 29 (for 1965-1966) show no difference in extensity of infection with D. ditremum in the three age groups, but indicated an increase in intensity with age; an inverse relationship between age and extensity of infection with D. osmeri and D. sp. Type II; a direct relationship between age and intensity of infection with D. osmeri and D. sp. Type I; and no change in intensity in the case of D. sp. Type II. Unlike kokanee, rainbow trout showed a marked difference in overall extensity among the various age groups (Table 30). None of the one or two year old specimens was infected; 30% of the 3-year olds carried plerocercoids; and all but one (98%) of the 5-7-year old groups were infected. Intensity followed the same pattern, ranging from zero in the 1-year and 2-year classes to a mean of 77.2 in the 7-year old category. The most heavily infected fish examined was a 7-year old 18-pound trout from which 109 plerocercoids of six species (including all

species found in the area except D. latum) were recovered.

The 1965-1966 data on trout (Table 31) show that both the extensity and the intensity of infection with the species which most frequently occurred (D. dendriticum, D. ditremum, D. cordiceps, and D. osmeri) increased with the age of the host.

Data for walleye and northern pike are recorded in Table 32 and Table 33; a relationship between age and infection similar to that in trout, is indicated for both.

Season and infection in Kokanee from Kootenay Lake

The data obtained showed no seasonal difference in the extensity of infection, but variations in intensity which seemed to have a seasonal bias have been noted (Table 34).

Since a direct relationship exists between the age of kokanee and the intensity of infection, variations in intensity due to the age composition of the samples examined at different times of year, might be inadvertently attributed to seasonal causes. In cases in which older fish make up a high percentage of the sample, a higher intensity has been noted. The high intensity recorded during the July-September period (Table 35), seems to be directly related to the high percentage of 3-year old specimens (75% of 731 specimens during that period, compared with 8% of 182 specimens during the April-June period, and 5% of 154 examined during the January-March period). Likewise, 2-year old specimens accounted

for 88% of the January-March sample, but only 48% of the April-June specimens, thus probably contributing to the lower overall average intensity during the latter period.

A genuine seasonal pattern, however, did seem to be present. In Fig. 30, the intensity for each age group is analyzed and plotted separately, thus avoiding any variation due to age differences. The intensity of infection in 1-year old specimens remained constant during spring and early summer, but showed an increase in August. The samples obtained during the winter and early spring months were inadequate to give a proper picture of any variations at that time of year.

Adequate numbers of 2-year old specimens were examined each month. A relatively small unexplained decrease in intensity occurred in March, followed by a marked rise in the level of infection during the summer period, suggesting that most of the infections might have been acquired at that time of year.

Three-year old specimens were obtained in sufficient numbers only during August and September. No meaningful conclusion can therefore be derived from the observed variations.

Year to year variations in infection

Some year-to-year fluctuations have been observed in the extensity or the intensity of infection (or both) in all five species of host, but these were most clear-cut in kokanee, in which there was a progressive decrease in intensity during the study period.

The intensity of infection decreased from 26.2 in 3-year old fish from the 1960 year class (3 years old in 1963) to 16.6 in 3-year old specimens from the 1963 year-class (3 years old in 1966) (Fig. 31). Similarly, the intensity in 2-year old specimens decreased from 20.8 in the 1961 year-class (2 years old in 1963) to 6.7 in the 1964 year-class (2 years old in 1966). Basically the same pattern was found in 1-year old fish from the 1962 to the 1965 year-classes.

Effects of the parasite on the host

Infected fish seemed to suffer no ill effects. All specimens were apparently in good condition, even when quite heavily infected. In many cases of heavy infection in kokanee and trout, a moderate adhesion of the viscera to the parietal peritoneum in the region adjacent to the stomach was present, but this condition had no apparent effect on the fish. Some kokanee with visceral adhesions had fully developed ovaries and were easily stripped of eggs.

Tissue-invading plerocercoids of trout (D. cordiceps, D. dendriticum), but not D. latum in pike or walleye, were generally encapsulated by a dense connective tissue cyst of host origin (Fig. 32). In some cases, a pus-like fluid was associated with such parasites. Connective tissue repair was evident in some histological sections through areas adjacent to these encysted worms.

Ecology of the plerocercoids occurring in Kootenay Lake.

There can be little doubt that at least some of the species of copepods present in Kootenay Lake are involved in the life-cycles of the species of Diphylllobothrium occurring in the salmonids of the lake. Several species of Cyclops and Diaptomus are known to serve as the natural first intermediate host of Diphylllobothrium, and the results of experiments (conducted during the present study) in which coracidia were fed to several species of copepods, strongly suggest that Cyclops bicuspidatus, one of the most common species on the lake (Zyblut, 1966) is a suitable host for the plerocercoids of D. dendriticum and D. ditremum, and so may be one of the main vectors.

However, none of the several thousand copepods examined, including Cyclops bicuspidatus, was infected; but this result might be due to the small size of the sample examined (5,000 to 8,000). In a large lake such as Kootenay, which supports inconceivably large numbers of copepods, a sample of this size is definitely not adequate to give a true picture of the extent of proceroid infection.

The general pattern of infection observed in the three salmonids may be explained on the basis of their feeding habits, and especially on the extent to which they normally feed on copepods. Kokanee are small fish, usually 18 to 28 cm. (7 to 11 in.) in length, which remain predominantly plankton-feeders throughout their entire life-span. Infections with Diphylllobothrium plerocercoids are

therefore undoubtedly a direct result of feeding on infected copepods.

The main source of infection of rainbow trout appears to be different. Trout do not normally feed on copepods, but do take in a fair quantity of these organisms along with their normal diet of insects, other invertebrates, and small fish. Unlike kokanee, rainbow trout change their food habit with age. Larkin et al (1957) have shown that the larger rainbow trout of Kootenay Lake are piscivorous; and Cartwright (1961) has indicated that this shift to a piscivorous diet, which includes kokanee as a principal item, occurs most predominantly after three years of age. The stomach contents of trout examined in the present study support this observation.

A close relationship exists between this shift in feeding habit of Kootenay Lake rainbow trout and the incidence of infection with Diphylllobothrium plerocercoids, for no trout below the age of three years was found infected. The nine infected three-year old specimens were quite large (42 to 65.8 cm.) and were capable of preying on kokanee. Trout apparently acquire their infections by eating kokanee.

Although the number of dolly varden char examined was small and the specimens were not aged, the pattern of infection closely parallels that in trout, and suggests the same dependence on kokanee. Infections increased directly with size, and only the larger speci-

mens were infected. The lower general incidence of infection in char may be due to the relatively smaller number which are large enough to feed on kokanee.

Unsworth (1944), Hickey and Harris (1947), and Vik (1957), all discuss the apparent importance of the stickleback (Gasterosteus aculeatus) as a vehicle for the transport of plerocercoids to trout. Vik found that no trout less than 25 cm. in length harboured the plerocercoids of Diphyllbothrium norvegicum, and Unsworth and Hickey and Harris found that only trout 20 cm. and over were parasitized by a species of Diphyllbothrium occurring in the British Isles. These authors noted that the distribution of Diphyllbothrium plerocercoids corresponded to the distribution of sticklebacks. They did not investigate the importance of sticklebacks in the food of trout, but there seems to be little doubt as to whether these small fish were indeed utilized by larger trout as an important article of food.

The smaller minimum size of infected trout reported by these workers (20-25 cm. versus 42 cm. in this study) might be explained by the size of the carrier host (sticklebacks are 2.5 to 7.5 cm. in length; the smallest kokanee examined was 14.8 cm.).

If trout acquire their infection by eating kokanee, the question of the mechanics of transport of plerocercoids of D. dendriticum and D. cordiceps may be raised. D. dendriticum plerocercoids were found in only four out of 1154 kokanee, and D. cordiceps

was never found in this host. How then may their presence in trout be explained?.

It is possible that the transport or the intermediate host of these species may not be kokanee, but some other organism which is eaten by trout but not by kokanee. A second possibility is that the juvenile forms of these species may be transported by kokanee to trout, the intermediate host, in which development into typical D. dendriticum and D. cordiceps plerocercoids occurs.

The first alternative seems rather unlikely since as far as is known, the arthropods which constitute the main source of food for trout prior to their shift to the predatory habit, have never been reported to be involved in Diphyllbothrium life-cycles. As Fraser (1960c) has pointed out, some of "these organisms may act as mechanical carriers of infected copepods" within their stomachs. It is not likely, however, that this would be the source of trout infection, since the size and age categories of trout which feed predominantly on such organisms were free of plerocercoids.

The second possibility seems more likely. Vik (1957) has shown that despite the basic similarity in histological structure between his Stage I and Stage II plerocercoids of D. norvegicum obtained from sticklebacks and rainbow trout respectively, the Stage I larvae (juvenile forms) were incapable of infecting any of the definitive hosts used in his experiments. Although

direct evidence is lacking, Diphyllbothrium sp. Type II, which resembles D. dendriticum in some respects (see Taxonomy), may be a juvenile form of D. dendriticum. Four feeding experiments carried out with this species of larva produced negative results, suggesting that the larvae might be juvenile forms which are not infective to the final host. On the other hand, the negative results could be due to the small number of worms used in each experiment (4-6), or to the unsuitability of the hosts used (two dogs and a gull).

The possibility that some other small forage fish might be involved in the transmission of D. dendriticum and D. cordiceps should not be overlooked. Bangham and Adams (1954) found Diphyllbothrium species in mountain white-fish (Prosopium williamsoni (Girard)) from Wood Lake and Grave Lake, British Columbia, both of which belong to the same drainage system (Columbia) as Kootenay Lake. Although none of the 53 mountain white-fish examined in the present study was infected with Diphyllbothrium plerocercoids, the sample was too small to suggest that in Kootenay Lake, this species of fish is not infected.

Examination of Mammals

None of the 13 mammals autopsied was infected with Diphyllbothrium. These included 2 black bears (Ursus americanus), 1 lynx (Lynx canadensis), 1 bob-cat (Lynx rufus), and 1 cougar (Felis

concolor), all shot in early summer; and 8 otters (Lutra canadensis) trapped in early winter. In addition, the 21 dogs and 19 cats obtained from the Kootenay Lake district for experimental studies, were Diphylllobothrium-free when treated with Nemural shortly after they were acquired. Two human infections were found during the study: the first was in a 19-year old female; the other in a 54-year old male, both residents of Nelson.

Examination of Birds

Table 36 shows that out of the 15 species of birds autopsied, only 4 species, 3 species of gulls and a merganser were infected, and that 33.3% of the 222 specimens examined were carrying Diphylllobothrium sp. However, most of the other birds were too small to feed on kokanee to any extent, and were collected in winter or early spring, when infections in birds were relatively low.

The mean overall incidence of infection among the four infected species from 1963 to 1965 was 40.4% (Table 37). This varied considerably during the 3-year period, decreasing from 77.8% of 45 birds in 1963 to 12.4% of 89 specimens in 1965. Approximately 57% of the 112 adult birds from these four species were carrying Diphylllobothrium, but only 14% of the 71 immatures (yearling mergansers; yearling or two year old gulls), were infected. This difference in infection might be due in part to the time of year when most of the young birds were collected - during spring and summer. At that

time of year, yearling gulls were usually present in relatively large numbers, and tended to concentrate and feed in the vicinity of the Nelson garbage dump.

Table 38 shows that the extensity of infection varied from month to month, a higher extensity occurring during September and October than during other months when comparable samples were examined.

The usual intensity of infection varied from 1 to 24 worms, but in two cases, over 100 specimens of Diphyllbothrium were recovered. Most of the worms were immature or bore only a few mature proglottids. In cases of heavy infection, the great majority showed little development beyond the plerocercoid stage. These immature specimens were not identified. One to three mature worms were found in 39 of the 74 infected birds; 41% of these were identified as D. dendriticum, and 59% as D. ditremum. No indication of host-specificity was observed for either species; both occurred in the four species of hosts, and in several cases, simultaneously in a single host. The frequencies of occurrence of the gravid specimens in the four hosts are shown in Table 39.

Ecology of the adult worms found in the Kootenay Lake area

The data obtained in this study leave little doubt that the most important sources of infection for natural definitive hosts are the tens of thousands of kokanee which spawn and die in late

summer and early autumn every year. At this time of year, thousands of gulls congregate in the vicinity of the spawning beds (Fig. 3), especially those at the northern extremity of the lake, to feed on dead and dying kokanee. Other birds, bears, bob-cats, and probably other mammals, feed heavily on these fish.

Since spawning kokanee are 100% infected, and the intensity of infection is relatively high, a large number of plerocercoids must be consumed by these potential definitive hosts. In one herring gull, shot at the Meadow Creek spawning ground in 1964, well over 100 specimens of Diphyllobothrium were recovered, most of which were small and had hardly progressed beyond the plerocercoid stage.

The importance of gulls in the dissemination of D. ditremum and D. dendriticum in the Kootenay Lake area is obvious, these being the only species found in these hosts. Approximately 90% of the gulls examined at this time of year were infected with fully mature D. ditremum and D. dendriticum which, according to experimental results, normally reach maturity and begin shedding eggs in 6 to 8 days after infection. If this reflects the actual percentage of infected gulls in the population at this time of year, and if a conservative estimate of the gull population is 2,000 to 3,000 birds, then millions of Diphyllobothrium eggs must be voided into the lake in the droppings of these birds during this period.

Spawning kokanee are 100% infected with D. ditremum plerocercoids which make up the bulk of plerocercoids found in this

species of fish. Since there is no other source of such abundant and readily available infective material in the area, and since most of the gulls emigrate in late fall and do not begin returning in large numbers until the following summer, it is very likely that massive infections of these birds with D. ditremum and extensive transference of D. ditremum eggs to the lake occur only once per year, during late summer to early fall. Even though the data pertaining to the monthly occurrence of gravid D. ditremum in infected birds (Table 40) are scant and so do not allow a definite conclusion, they suggest that this might indeed be the case. Only four and seven percent of the birds harboured gravid D. ditremum in July and August; forty and twenty-four percent were carrying this species in September and October respectively. It appears that gulls acquire the bulk of their D. ditremum infections through feeding on spawning kokanee, but also acquire subsidiary infections from the discarded remains of kokanee, trout, and char (Fig. 33a).

The data in Table 40 suggest a less extensive rise in infection with D. dendriticum in the fall, indicating a lesser dependence on spawning kokanee. Since typical D. dendriticum plerocercoids are abundant in trout, but virtually lacking in kokanee and char, the former seem to be the main link in the cycle of transmission (Fig. 33b). The main source of infection with D. dendriticum is most probably trout viscera discarded on the lake and on garbage dumps.

Other species of birds and/or mammals must be involved in the life-cycles of some of the species of Diphyllbothrium found in the locality. D. osmeri, though the second most abundant plerocercoid found in kokanee, was not found in natural infections of gulls (nor mergansers), and seemed to be ill-adapted to experimentally infected gulls from which the worms were often spontaneously evacuated within a week of feeding and from which mainly immature specimens were obtained. Neither did they adapt well to cats, but grew satisfactorily in dogs (58.8% of 16 feedings).

These findings indicate that some other species of host or hosts (probably a mammal) is involved in the natural life-cycle of this species (Fig. 33c). It is unfortunate that no bear was captured during the kokanee spawning period when the opportunity for infection with this species (as well as with D. ditremum) is optimal. Both of the bears autopsied were captured in early summer, and by then might have lost any infection acquired during the previous fall. Since bears are known to be definitive hosts of other species of Diphyllbothrium (D. latum, D. ursi), their probable involvement in the life-cycle of D. osmeri cannot be discounted. As far as is known, the natural definitive host (or hosts) of D. osmeri has never been found, even in localities where the plerocercoids are relatively abundant (Kuhlow, 1953b; Chizova et al, 1962; Wikgren, 1964; Vik, 1964b).

The definitive host (or hosts) of D. cordiceps was not

found, but the fact that the plerocercoid was found in trout but not in kokanee, probably suggests a mechanism of transmission similar to that of D. dendriticum (Fig. 33d).

The plerocercoid of D. latum was very rare. However, because this is usually a tissue-invading plerocercoid, and since the flesh of many large rainbow trout was not available for examination, the probability of a higher incidence should not be overlooked. Because the worm recovered from one of the two human infections found during the study was identified as D. latum, and was traced to trout, a probable human host in the cycle is suggested.

Year to Year variations in infection of Kokanee and Gulls

The progressive decrease in the intensity of infection observed in kokanee during the study period (Fig. 31) seems to be paralleled by a similar decrease in the extensity of infection in gulls. Although there was undoubtedly an authentic reduction in extensity among these birds over the years, the age composition of the samples collected each year had an important effect on the mean annual extensity of infection, since differences in extensity due to age have been recorded. Immature birds made up 15.6% of the 1963 sample, 28.6% of the 1964 specimens, and 56.2% of the 1965 collection.

No reasonable explanation of the reduction of infection levels in kokanee could be offered. In his effort to control D. norvegicum infection in the trout of a Norwegian lake, Vik (1965) had demonstrated the effectiveness of destroying the definitive

hosts, Larus canus. Destruction of these birds and their eggs resulted in a drastic reduction in the extensity of infection from 57% in 1950 to 4.5% in 1964, with corresponding reductions in intensity from 27 plerocercoids to 0.2 worms per fish. During the present study slightly over 200 actual and potential definitive hosts were eliminated, and 58 fully matured adult worms removed from the ecosystem. But because of the immense number of gulls present in the area, especially during the kokanee spawning period, such activities have very likely not affected the level of infection in kokanee.

Similarly, the several hundred kokanee with over 20,000 plerocercoids, autopsied by the author and so made unavailable to potential hosts, might not have contributed to any possible reduction in the extensity of infection among gulls. The most important probable cause of any genuine decrease in such extensity might be the greatly increased traffic along the road which passes alongside the Meadow Creek spawning ground since 1964 when construction of the Duncan River dam north of the lake began. The traffic might have scared away many potential definitive hosts from the area thus reducing their probability of infection.

The increased awareness among residents of the district of the potential risk of human infection, and of infection of some domestic animals, is one factor which might have contributed to some extent in containing the spread of infection at times other than during the kokanee spawning runs. Burning or burying fish

offal is now generally practised by resort operators and by anglers.

Public Health

The distribution of plerocercoids in the organs of their hosts seems to have some practical public health implications. Human infections through kokanee is probably unlikely since only as insignificant percentage of the worms was found in the musculature of this species of fish.

Trout, however, can be considered a probable source of human infection since 40.5% of the infected specimens or 21.7% of all trout examined (Tables 15 and 16) carried plerocercoids in the flesh; and trout, smoked by methods which may not always kill encysted larvae, is used by many residents of the area. The case-history of one of the two human infections in that area points to the consumption of smoked trout some months earlier as the probable source of the infection.

As far as could be ascertained, the two cases of human diphyllobothriasis reported in this study, constitute the only records for the Kootenay district. It is possible that the incidence is higher, but unrecognized, due to the absence of any symptoms in most cases. In the first case found, the worm had been mistakenly identified by others as beef tapeworm (Taenia saginata) "brought into the district in cheap New Zealand beef". The specimen has since been identified as D. latum.

A systematic stool examination of a significant sample of local residents, with a view to determining the extent of human infection, was proposed, but had to be called off.

Ecology of the species occurring in Iosegun Lake

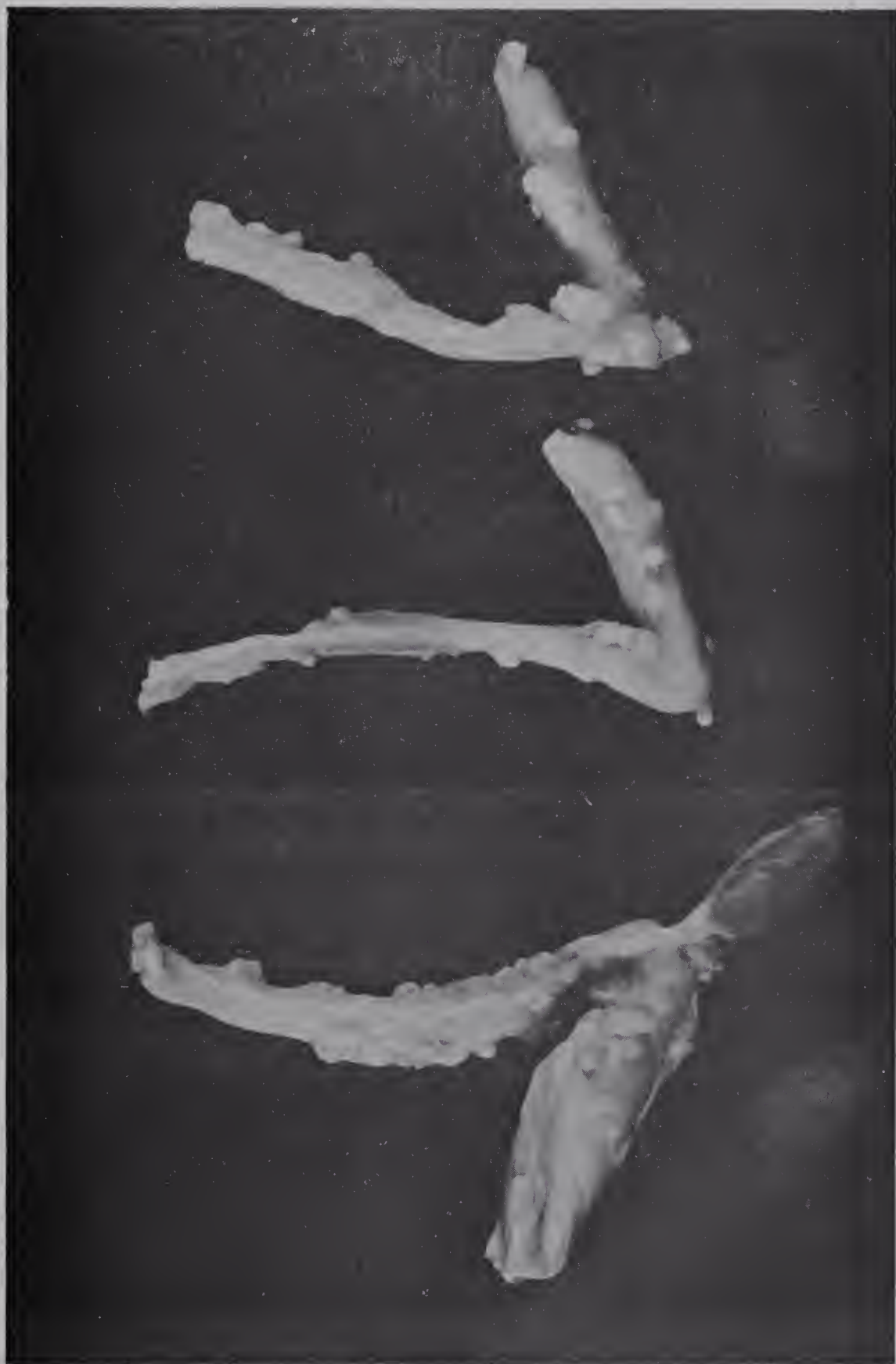
Little work has been done on the ecology of the species occurring in the pike and walleyes of Iosegun Lake. The pattern of infection, however, appears to be analogous to that of trout and dolly varden char from Kootenay Lake, with only the larger, primarily fish-eating specimens infected. Thus, a small fish intermediary between the copepod first intermediate host and the predatory fish, is indicated.

The relatively low intensity and/or extensity of infection may be due in part to that fact that this lake remains frozen over for about 6-7 months of the year, consequently limiting the period during which Diphylllobothrium eggs may enter the system with the excreta of the definitive host. The observed difference in extensity and intensity between pike and walleye may indicate that the plerocercoids have a shorter life span in pike than in walleye. Nicholson (1932) postulated a seasonal death of D. latum plerocercoids in the pike of Lake Winnipeg, Manitoba, as an explanation of the lower incidence of infection and the smaller size of plerocercoids during the spring and early summer months. The exact situation in pike and walleye from Iosegun Lake was not determined, however.

All six birds (none of which were very likely hosts for D. latum) shot on the lake, were Diphylllobothrium-free. No other possible definitive host was available for examination, so no definitive host has been established for the parasite in this locality. However, as stated in the Introduction of this study, an adult Diphylllobothrium was recovered from a dog owned by a trapper who lives near the lake and normally fed raw fish to his dogs. No periodic mass die-off of infected fish occurs at Iosegun Lake, so that a lower extensity of infection than that in Kootenay Lake would be expected among wild animal hosts. Because the plerocercoids are almost exclusively in the flesh, and not in the viscera which are discarded by man and so might still be available to such animals, only if animals such as bears and crows should feed on spawning or dead fish would the plerocercoids be transmitted. The likelihood of a human link in this chain is suggested. Man and/or domestic animals such as dogs, to which raw fish is fed, may be the most important definitive hosts involved.

The author has been reliably informed that until recently, raw sewage and refuse from the nearby oil trailer camps and from a recently disbanded Indian community, were emptied into a stream which drains directly into the lake. Although no definitive host has been found, these conditions suggest a possible mechanism for propagating and maintaining the infection.

Fig. 29 - Encysted plerocercoids on the stomach serosa
of 3-year old spawning kokanee.



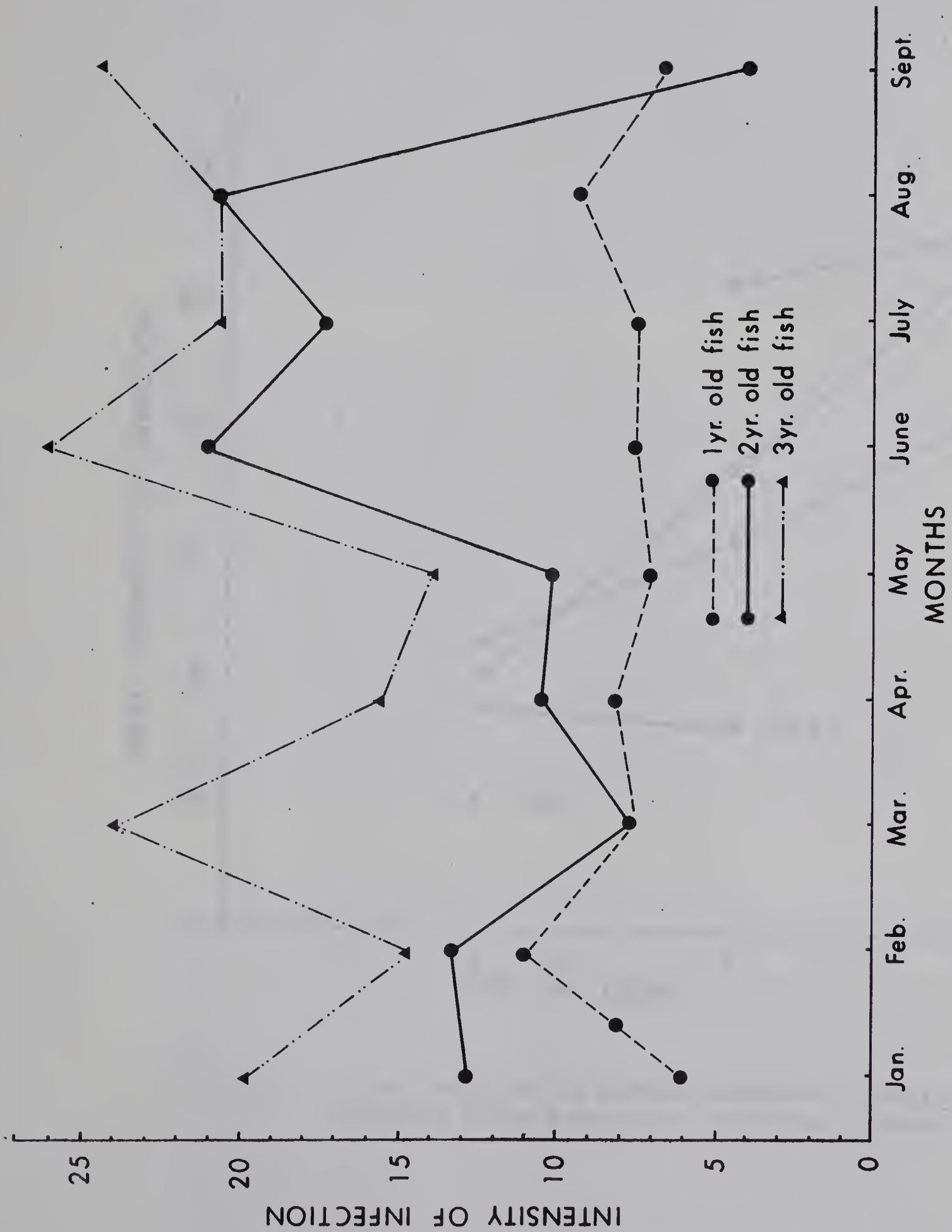


FIG. 30 - SEASONAL VARIATION IN THE INTENSITY OF INFECTION IN KOKANEE

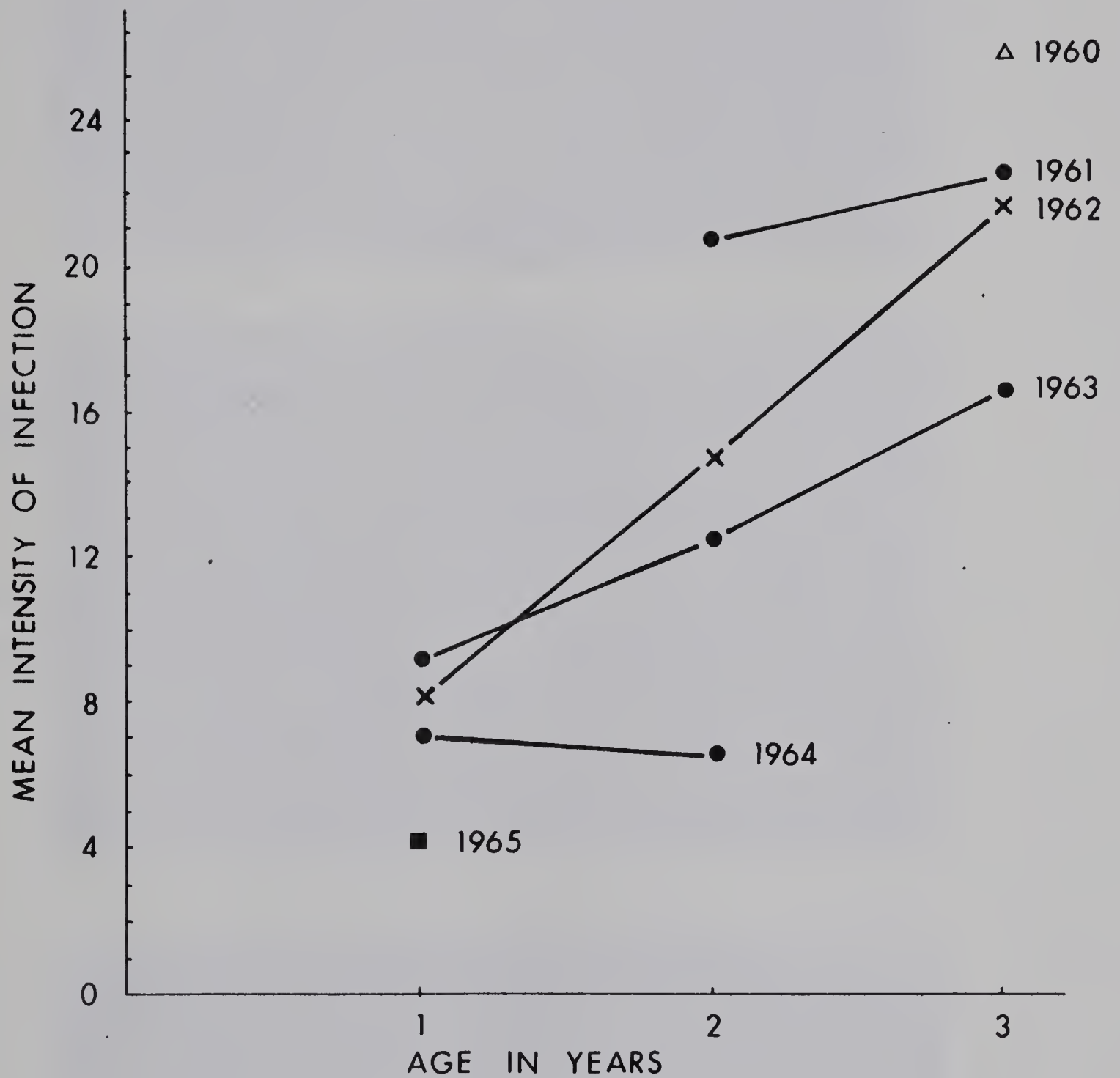
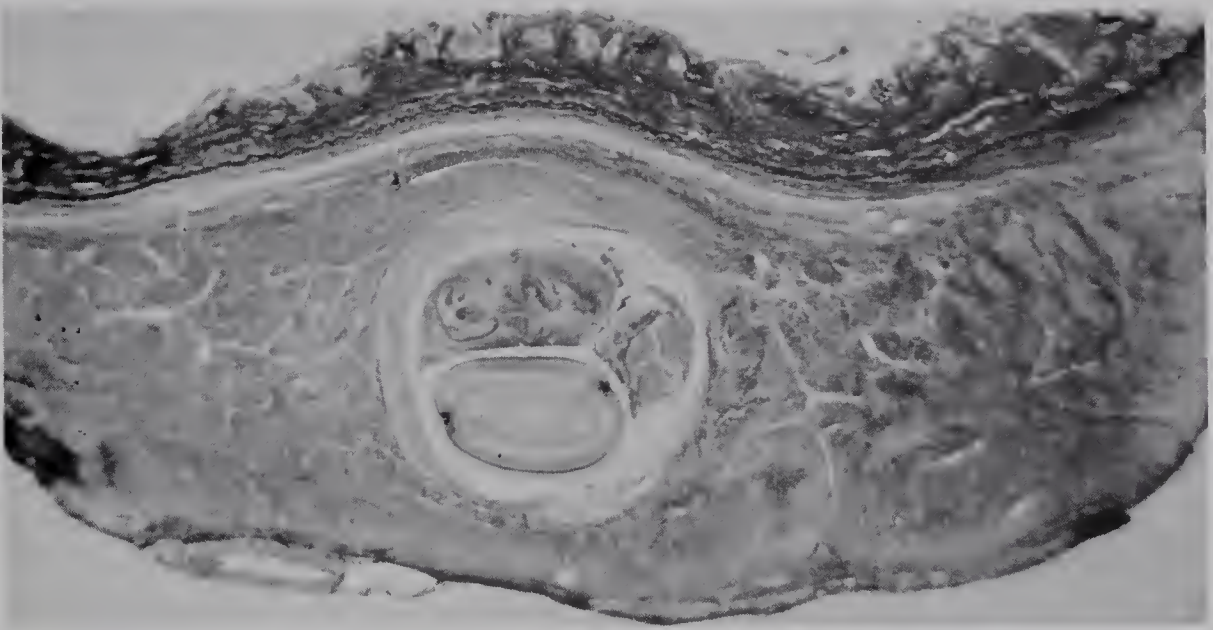


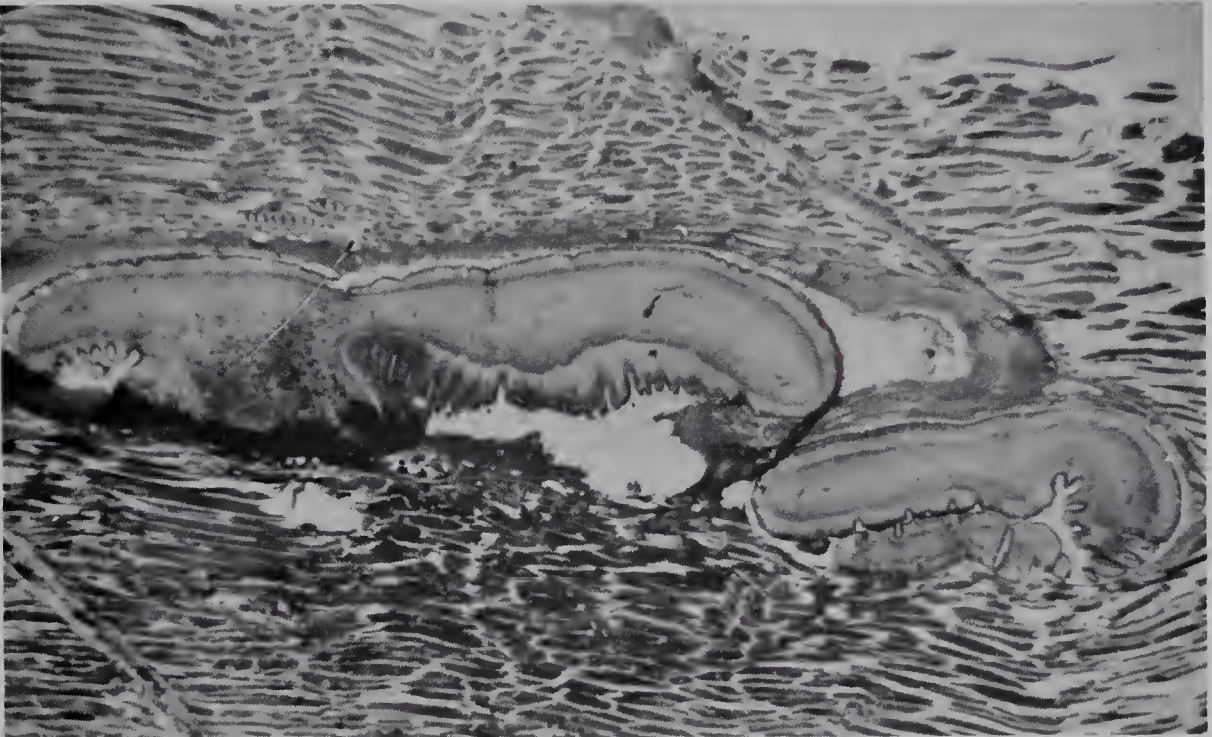
FIG. 31 -- YEAR-CLASS ANALYSIS SHOWING PROGRESSIVE YEAR-TO-YEAR DECREASE IN THE INTENSITY OF INFECTION IN KOKANEE.

Fig. 32 - Plerocercoids in the musculature of rainbow trout;
(note connective tissue "capsule" around parasites).

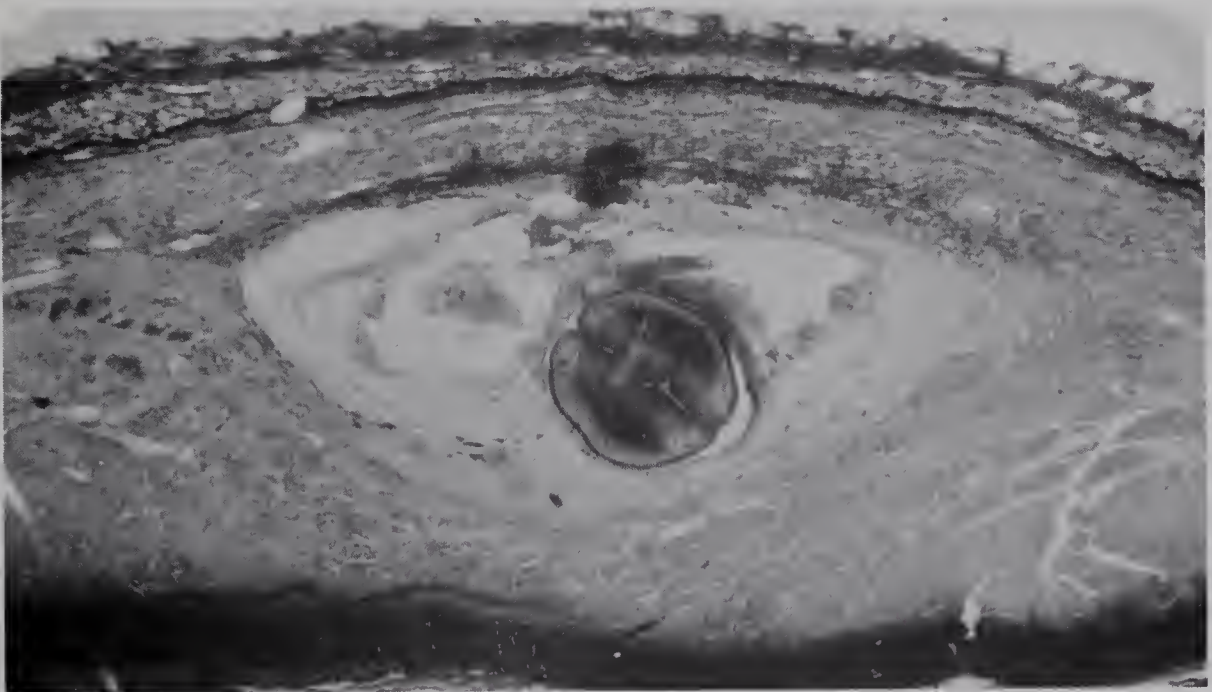
- a - transverse section through worm in
epaxial muscle.
- b - oblique section of worm in hypaxial muscle.
- c - transverse section through scolex of worm
in muscle of stomach wall.



a



b



c

FIG. 33

Probable Life-Cycles of four species of Diphyllbothrium
in the Kootenay Lake area.

- a. D. ditremum
- b. D. dendriticum
- c. D. osmeri
- d. D. cordiceps

() - presumed natural host .

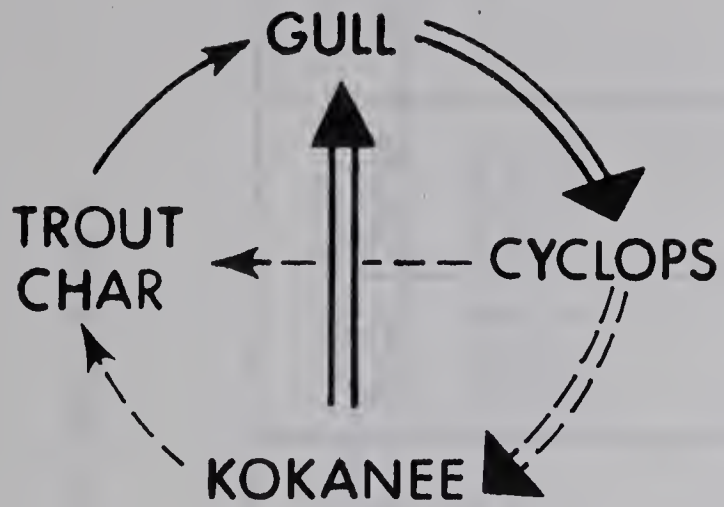
(()) - presumed host of juvenile plerocercoid (not demonstrated; see text).

==> - probable major route (experimentally demonstrated) .

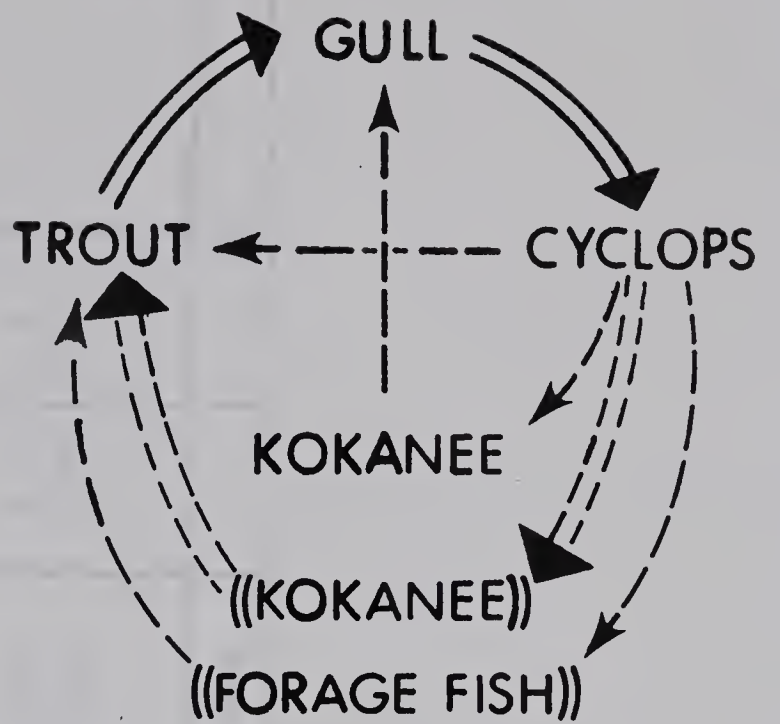
—> - probable supplementary route (experimentally demonstrated) .

==> - probable major route (not tested) .

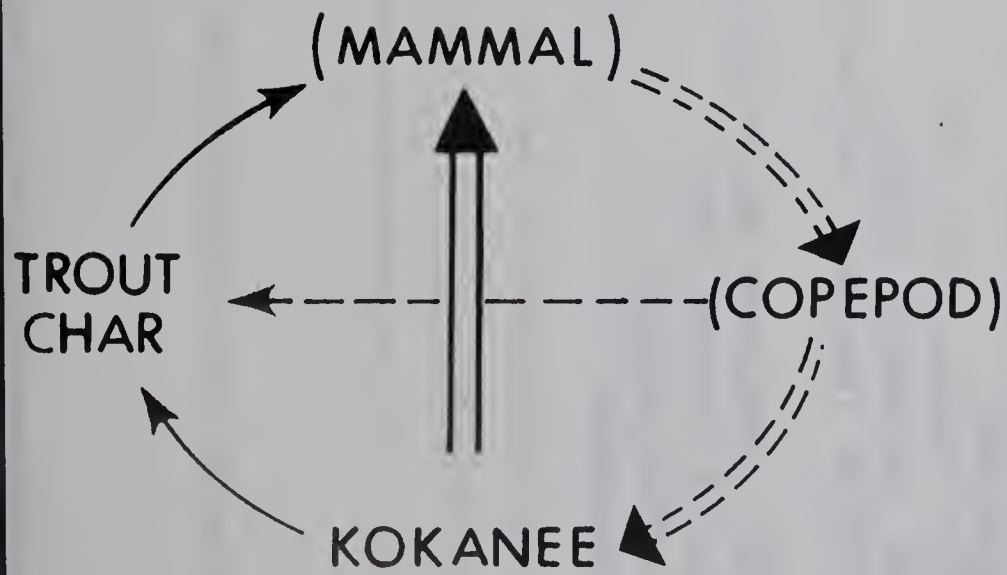
--> - probable supplementary route (not tested) .



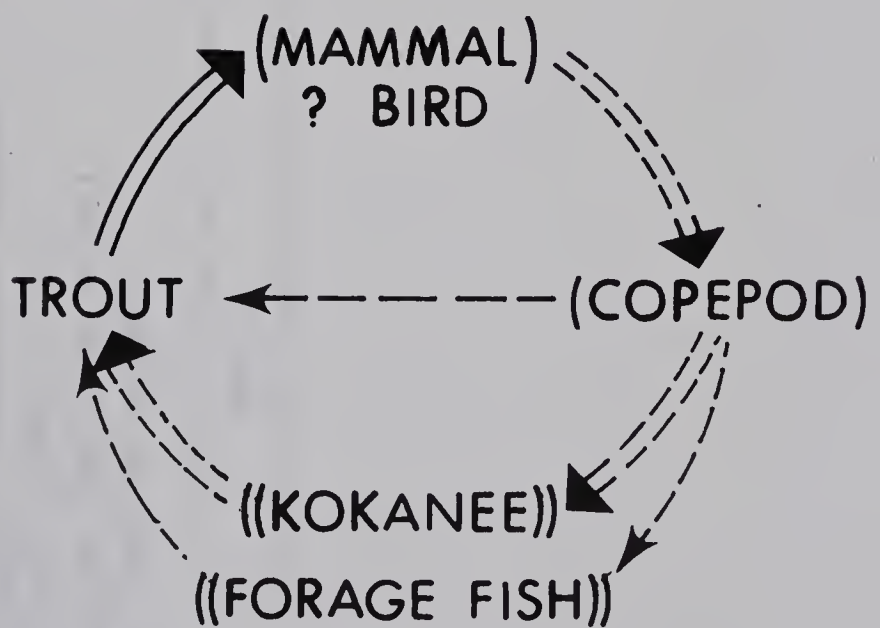
a.



b.



c.



d.

Table 13

Extensivity and intensity of infection in fish examined 1963-1966.

Species of Fish	No. examd	Infected		Intensity		No. of D. sp.
		No.	%	Mean	Range	
<u>Oncorhynchus nerka</u> (Kokanee)	1154	1149	99.5	17.8	2-66	5
<u>Salmo gairdneri</u> (Rainbow trout)	157	84	53.5	59.8	2-109	7
<u>Salvelinus malma</u> (Dolly Varden char)	50	10	20.0	17.3	7-44	5
<u>Prosopium williamsoni</u> (Mountain whitefish)	53	0				
<u>Lota lota</u> (Burbot)	62	0				
<u>Ptychocheilus oregonense</u> (Northern squawfish)	9	0				
<u>Mylocheilus caurinus</u> (Peamouth chubb)	7	0				
<u>Micropterus salmoides</u> (Largemouth bass)	2	0				
<u>Lepomis gibbosus</u> (Sunfish)	1	0				
<u>Acipenser transmontanus</u> (White sturgeon)	1	0				
<u>Catostomus catostomus</u> (Longnose sucker)	10	0				
<u>Esox lucius</u> (Northern pike)	65	9	13.8	1.7	1-4	1
<u>Stizostedion vitreum</u> (Walleye pike)	32	16	50.0	5.8	1-37	1
<u>Perca flavescens</u> (Yellow perch)	9	0				
Total	1612	1268	78.6			

Table 14

Distribution of the seven species of plerocercoids among the 5 species of hosts, 1965-1966

Fish species	% of total plerocercoids of						
	<u>D. dend.</u>	<u>D. ditrem.</u>	<u>D. osmeri</u>	<u>D. cord.</u>	<u>D. latum</u>	<u>D. sp.I</u>	<u>D. sp.II</u>
<u>Oncorhynchus nerka</u>	0.1	82.1	14.1	-		2.2	1.5
<u>Salmo gairdneri</u>	13.6	63.9	11.0	4.0	0.2	5.3	2.0
<u>Salvelinus malma</u>	2.0	80.0	12.0	-		4.0	2.0
<u>Esox lucius</u>	-				100		
<u>Stizostedion vitreum</u>	-				100		

Table 15

Percentage of fish infected with one or more species
of plerocercoids in various organs 1963-1966

Species of Fish	% of specimens with plerocercoids in/on						
	Upper Digest. Tract	Spleen	Liver	Gonad	Swim- bladder	Muscle	Other locat- ions
<u>Oncorhyncus nerka</u>	99.5	3.2	4.5	8.6	0.7	1.7	9.6
<u>Salmo gairdneri</u>	53.5	10.2	21.7	21.7	8.9	21.7	28.0
<u>Salvelinus malma</u>	20.0						
<u>Esox lucius</u>	1.5					12.3	
<u>Stizostedion vitreum</u>	25.0	3.1	3.1	3.1		40.6	9.6

Table 16

Percentage of infected fish with plerocercoids of
one or more species in various organs

Species of Fish	% of infected fish with plerocercoids in/on						
	Upper Digest. Tract	Spleen	Liver	Gonad	Swim- bladder	Muscle	Other locat- ions
<u>Oncorhyncus nerka</u>	100	3.3	4.5	8.7	0.8	1.7	9.6
<u>Salmo gairdneri</u>	100	19	40.5	40.5	16.7	40.5	52.4
<u>Salvelinus malma</u>	100						
<u>Esox lucius</u>	11.1					88.9	
<u>Stizostedion vitreum</u>	50	6.2	6.2	6.2		81.2	18.9

Table 17

Distribution of plerocercoids of one or more species in the organs of fish hosts, 1963-1966

Species of Fish	Percentage of Plerocercoids Encysted in or on											Percentage of Plerocercoids Unencysted on or in										
	Stomach (muscle)	Stomach (serosa)	PGF	Caecae	Spleen	Liver	Gonad	Swim-bladder	Muscle	Other organs	Total encysted	Stomach (muscle)	Stomach (serosa)	PGF	Caecae	Spleen	Liver	Gonad	Swim-bladder	Muscle	Other organs	Total unencysted
<u>Oncorhynchus nerka</u>	1.0	40.3	6.5	13.9	0.2	0.2	0.3	*	*	0.5	62.9	4.4	13.9	14.1	2.8	0.1	0.2	0.7	*	0.1	0.8	37.1
<u>Salmo gairdneri</u>	1.7	27.6	9.3	12.2	0.2	0.6	0.4	0.3	1.6	1.1	55.0	3.7	13.7	18.8	4.9	0.2	0.5	1.5	0.2	0.2	1.2	45.0
<u>Salvelinus malma</u>	4.5	54.2	5.2	15.5							79.3	5.2	5.7	9.7								20.7
<u>Esox lucius</u>											-			6.7						93.3		100
<u>Stizostedion vitreum</u>	9.8				1.1	1.1				1.1	13.1	2.2	2.2	9.8				3.2		56.5	13.0	86.9
	Total Percentage Encysted											Total Percentage Unencysted										
	42.0											58.0										

Total Percentage in Viscera 99.0

*Less than 0.1%

Total Percentage in Muscle 1.0

Table 18

Frequency of infection in the organs of infected kokanee and rainbow trout 1965-1966*

Parasite Species	Fish Species	Percentage of fish infected in or on						
		Digest. tract	Spleen	Liver	Gonads	Swim- bladder	Muscle	Other locations
<u>D. dendriticum</u>	<u>Oncorhynchus nerka</u>						0.8	
	<u>Salmo gairdneri</u>	100	14.3	31.4	20	17.1	8.6	8.6
<u>D. ditremum</u>	<u>Oncorhynchus nerka</u>	100	0.8	2.3	4.8	0.2		3.4
	<u>Salmo gairdneri</u>	100			28.6			45.7
<u>D. osmeri</u>	<u>Oncorhynchus nerka</u>	16.2	0.4	1.3	1.5	0.6		2.3
	<u>Salmo gairdneri</u>	17.1			11.4			5.7
<u>D. cordiceps</u>	<u>Oncorhynchus nerka</u>							
	<u>Salmo gairdneri</u>	20		8.6			42.8	8.6
<u>D. latum</u>	<u>Oncorhynchus nerka</u>							
	<u>Salmo gairdneri</u>							5.7
<u>Diphylllobothrium sp.I</u>	<u>Oncorhynchus nerka</u>	4						0.4
	<u>Salmo gairdneri</u>	5.7						5.7
<u>Diphylllobothrium sp.II</u>	<u>Oncorhynchus nerka</u>	5.5						1.3
	<u>Salmo gairdneri</u>							8.6

*Based on 476 kokanee and 35 rainbow trout

Table 19

Extensivity and Intensity of infection in kokanee and rainbow trout from six Kootenay Lake zones, 1963

Fish Species	Zone 1		Zone 2		Zone 3		Zone 4		Zone 5		Zone 6	
	No. examd	Intns. Extns.	No. examd	Intns. Extns.	No. examd	Intns. Extns.	No. examd	Intns. Extns.	No. examd	Intns. Extns.	No. examd	Intns. Extns.
<u>Oncorhynchus nerka</u>	105	98.1 19.1	40	97.5 20.3	53	98.1 19.8	44	97.8 22.1	59	100 20.0	50	100 24.3
<u>Salmo gairdneri</u>	6	50.0 55.4	6	66.7 56.9	8	50.0 56.0	3	66.7 54.2	4	50.0 50.9	-	-
Total	111		46		61		47		63		50	

Table 20

Extensivity and intensity of infection in kokanee and rainbow trout from six Kootenay Lake zones, 1964

Fish Species	Zone 1		Zone 2		Zone 3		Zone 4		Zone 5		Zone 6	
	No. examd	Intns. Extns.	No. examd	Intns. Extns.	No. examd	Intns. Extns.	No. examd	Intns. Extns.	No. examd	Intns. Extns.	No. examd	Intns. Extns.
<u>Oncorhynchus nerka</u>	88	100 17.8	29	100 18.0	30	100 20.3	49	100 19.2	46	100 19.2	40	100 22.1
<u>Salmo gairdneri</u>	20	45.0 60.8	19	47.4 68.5	12	50.0 69.8	9	44.4 63.9	6	50.0 62.2	-	-
Total	108		48		42		58		52		40	

Table 21

Size of host vs. infection
in Kokanee, 1963-1966

Class	No. examd	No. infctd	% infctd	Intensity	
				Mean	Range
14.7-17.7	40	40	100	5.9	3-13
17.8-20.8	316	313	99.1	17.9	2-66
20.9-23.9	387	387	100	18.4	3-58
24.0-27.0	150	149	99.3	18.3	6-59
27.1-30.1	50	49	98.0	20.0	6-52
30.2-33.2	19	19	100	21.2	9-28
33.3-36.3	45	45	100	16.9	6-34
36.4-39.4	63	63	100	18.9	10-34
39.5-42.5	64	64	100	16.8	7-41
42.6-45.6	13	13	100	17.8	10-29
45.7-48.7	7	7	100	16.1	10-25
Total	1154	1149	99.5	17.8	

Table 22

Size of Kokanee vs. infection with various species of plerocercoids, 1965-1966

Size class	No. of Specimens		<u>D. dendriticum</u>		<u>D. ditremum</u>		<u>D. osmeri</u>		<u>D. sp.I</u>		<u>D. sp.II</u>	
	Examd.	Infctd.	Extens.	Intens.	Extens.	Intens.	Extens.	Intens.	Extens.	Intens.	Extens.	Intens.
14.7-17.7	24	24			100	6.0	8.3	1.0				
17.8-20.8	106	106			100	11.0	10.4	9.0			8.5	1.4
20.9-23.9	148	148	2.0	2.0	100	12.1	4.0	13.7	2.7	6.0	4.7	4.8
24.0-27.0	84	83	1.2	1.0	98.8	13.6	10.7	13.6	7.1	7.0	13.1	5.0
27.1-30.1	20	20			100	14.2	15.0	14.0	25.0	10.0		
30.2-33.2	4	4			100	14.8	100	14.0			25	5.0
33.3-36.3	15	15			100	13.3	33.3	14.8	13.3	7.8		
36.4-39.4	29	29			100	14.0	20.7	14.8				
39.5-42.5	34	34			100	13.8	61.8	14.1	8.8	8.3		
42.6-45.6	7	7			100	14.2	100	14.2				
45.7-48.7	6	6			100	14	50	13.7				
	477	476	0.8	0.8	99.8	12.3	16.1	13.0	4.2	7.8	5.9	3.8

Table 23

Size of host vs. infection
in Rainbow trout, 1963-1966

Class	No. examd	No. infctd	% infctd	Intensity	
				Mean	Range
15.4-24.4	18	0	0		
24.5-33.5	21	0	0		
33.6-42.6	25	4	16.0	39.8	2-70
42.7-51.7	23	15	65.2	48.1	11-84
51.8-60.8	37	32	86.5	56.7	24-78
60.9-69.9	20	20	100	69.4	39-109
70.0-79.0	9	9	100	67.8	48-75
79.1-88.1	4	4	100	83.2	72-93
Total	157	84	53.5	59.8	

Table 24

Size of rainbow trout vs. infection with various species of plerocercoids, 1965-1966

Size class (cm.)	No. of Specimens		D. dend.		D. ditrem.		D. osmeri		D. cordiceps		D. latum		D. sp. I		D. sp. II	
	Exmd	Infctd	Extns.	Intns.	Extns.	Intns.	Extns.	Intns.	Extns.	Intns.	Extns.	Intns.	Extns.	Intns.	Extns.	Intns.
15.4-24.4	6															
24.5-33.5	5															
33.6-42.6	9	1	11.1	2.0	11.1	11.0										
42.7-51.7	7	5	71.4	6.7	71.4	31.4	14.3	7.0								
51.8-60.8	15	12	80.0	7.8	80.0	40.8	13.3	26.5	33.3	3.0	6.7	1.0				
60.9-69.9	7	7	100	8.6	100	38.2	28.6	21.7	57.1	6.5	14.3	2.0	28.6	33.0	28.6	13.0
70.0-79.0	7	7	100	8.0	100	42.7	14.3	23.0	42.9	6.0					14.3	14.0
79.1-88.1	3	3	100	8.0	100	44.3	33.3	22.6	100	6.7			33.3	38.0		
	50	35	59.3	7.7	59.3	35.9	16.9	21.7	25.4	5.3	3.4	1.5	5.1		5.1	13.3

Table 25

Size of host vs. infection in Dolly varden char
1963-1966.

Class	No. examd	No. infctd	% infctd	Intensity	
				Mean	Range
20.5-27.7	3	0	0		
27.8-35.0	8	0	0		
35.1-42.3	17	0	0		
42.4-49.6	9	3	33.3	21.3	7-44
49.7-56.9	9	4	44.4	20.2	13-18
57.0-64.3	4	3	75.0	16.0	11-26
Total	50	10	20.0	17.3	

Table 26
Size of host vs. infection in Walleye 1960 and 1965-1966

Class	No. examined			Extensivity of infection		Intensity of infection		
	1960*	1965-1966	Total	1960	1965-1966	1960	1965-1966	Range
22.4-26.7	2	1	3	0				
26.8-31.1	2	-	2	0				
31.2-35.5	4	4	8	0	75.0		1.0	1
35.6-39.9	19	5	24	52.6	0	5.9	0	1-10
40.0-44.3	18	16	34	88.9	62.5	9.9	7.3	1-42
44.4-48.7	3	6	9	100	50.0	10.7	5.3	5-18
Total	48	32	80	60.4	50.0	8.6	5.8	

*Examined by J.C. Holmes, Dept. of Zoology, University of Alberta.

Table 27

Size of host vs. infection in Northern pike,
1965 and 1966

Class	No. examd	No. infctd	% infctd	Intensity	
				Mean	Range
32.8-37.1	4	0			
37.2-41.5	2	0			
41.6-45.9	7	0			
46.0-50.3	19	2	10.5	1.0	1
50.4-54.7	26	6	23.1	1.5	1-3
54.8-59.1	5	1	20.0	4.0	4
59.2-63.5	2	0			
Total	65	9	13.8	1.7	

Table 28

Age of host vs. infection
in Kokanee, 1963-1966

Age Group (yrs.)	No. examd	No. infctd	% infctd (Extensity)	Intensity	
				Mean	Range
1	208	208	100	7.8	2-14
2	331	326	98.5	14.3	6-49
3	578	578	100	23.0	7-66
Total	1117	1112	99.6	17.8	

Table 29
Age of Kokanee vs. infection with various species of plerocercoids, 1965-1966

Age (yrs.)	No. of Specimens		<u>D. ditremum</u>		<u>D. osmeri</u>		<u>D. sp. I</u>		<u>D. sp. II</u>	
	Examd.	Infctd.	Extens.	Intens.	Extens.	Intens.	Extens.	Intens.	Extens.	Intens.
1	87	87	100	7.1	21.8	5.4	4.6	4.0	10.3	3.8
2	155	154	99.4	11.0	20.0	14.7	5.8	9.0	5.8	3.7
3	235	235	100	15.0	11.5	16.4	3.0	9.0	4.2	3.8
	477	476	99.8	12.3	16.1	13.1	4.2	7.8	5.9	3.8

Table 30

Age of host vs. infection
in Rainbow trout, 1963-1966

Age Group (yrs.)	No. examd	No. infctd	% infctd (Extensivity)	Intensity	
				Mean	Range
1	15	0			
2	30	0			
3	30	9	30.0	40.3	2-71
4	23	17	73.9	47.6	24-76
5	19	18	94.7	63.6	31-87
6	32	32	100	65.3	39-96
7	8	8	100	77.2	39-109
Total	157	84	53.5	59.8	

Table 31

Age of rainbow trout vs. infection with various species of plerocercoids, 1965-1966

Age (yrs.)	No. of Specimens		<u>D. dend.</u>		<u>D. ditrem.</u>		<u>D. cordiceps</u>		<u>D. osmeri</u>	
	Examd.	Infctd.	Extens.	Intens.	Extens.	Intens.	Extens.	Intens.	Extens.	Intens.
1	6	0								
2	6	0								
3	14	4	28.6	2.5	28.6	11.0	7.1	1.0		
4	10	8	80.0	6.2	80.0	14.4	10.0	3.0	20.0	16.0
5	6	6	100	9.1	100	48.3	33.3	6.5		
6	14	14	100	9.0	100	48.2	57.1	5.6	50.0	21.7
7	3	3	100	9.0	100	53.3	100	5.7	33.3	33.0
	59	35	59.3	7.7	59.3	35.9	25.4	5.3	16.9	21.7

Table 32

Age of host vs. infection in Walleye

Age Group (yrs.)	No. examd	Infected		Intensity		1960 Survey *			
						No. examd	Infected		Intensity
		No.	%	Mean	Range		No.	%	
1	-					-	-		
2	1	0				-	-		
3	1	0				3	0		
4	3	2	33.3	5	2-8	8	1	12.5	3
5	10	5	50	9	1-37	13	10	77.0	12.2
6	10	5	60	3.8	1-8	21	15	71.4	6.7
7	5	3	60	4.3	4-6	2	2	100	8.0
8	2	1	50	4	4	-	-	-	-
9	-					1	1	100	7.0
Total	32	16	50	5.8		48	29	60.4	8.6

*Conducted by J.C. Holmes - Dept. of Zoology, University of Alberta.

Table 33

Age of host vs. infection in Northern pike,
1965 and 1966

Age Group (yrs.)	No. examd	Infected		Intensity of infection	
		No.	%	Mean	Range
1	-	-			
2	7	0			
3	24	0			
4	26	7	27	1.6	1-4
5	8	2	25	2.0	1-3
Total	65	9	13.8	1.7	

Table 34

Monthly variations in the intensity of infection in Kokanee, 1963-1965

Month	No. of specimens examined				Intensity of infection			Mean overall intensity
	Total	1 yr.old	2 yr.old	3 yr.old	1 yr.old	2 yr.old	3 yr.old	
January	29	2	23	4	8.0	12.9	19.8	13.5
February	50	4	43	3	11.0	13.3	14.7	13.2
March	75	5	69	1	7.6	7.7	24.0	9.2
April	44	8	31	5	8.2	10.5	15.6	10.6
May	33	18	12	3	7.1	10.1	14.0	8.8
June	105	57	41	7	7.6	21.0	26.1	14.1
July	127	72	50	5	7.5	17.3	20.6	11.9
August	194	23	21	150	9.3	20.7	20.7	19.4
September	410	12	1	397	6.7	4.0	24.5	23.9
Total	1067*	201	291	575	7.7	13.8	23.3	17.9

* 4 specimens less than 1 year old are not included.

Table 35
Seasonal variations in the intensity of infection in Kokanee, 1963-1965

Period	No. of fish examined			% of sample			Intensity
	1 yr.	2 yr.	3 yr.	Total	1 yr.	2 yr.	3 yr.
January - March	11	135	8	154	7.1	87.7	5.2
April - June	83	84	15	182	45.6	46.2	8.2
July - September	107	72	552	731	14.6	9.8	75.6
Total	201	291	575	1067	18.8	27.3	53.9
							17.9

Table 36

Extensivity of infection in birds examined, 1963-1965.

Species	Number examined			Percent infected			
	1963	1964	1965	Total	1963	1964	1965
<u>Larus delawarensis</u> (ring-billed gull)	20	29	59	108	80.0	55.2	13.6
<u>Larus argentatus</u> (herring gull)	17	5	18	40	76.5	80.0	5.6
<u>Larus californicus</u> (California gull)	-	9	11	20	-	66.7	18.2
<u>Mergus merganser</u> (common merganser)	8	6	1	15	75.0	16.7	0
<u>Podiceps auritus</u> (horned grebe)		13		13			
<u>Podiceps caspicus</u> (eared grebe)		1		1			
<u>Podiceps grisegena</u> (red-necked grebe)			3	3			
<u>Aechmophorus occidentalis</u> (western grebe)			1	1			
<u>Fulica americana</u> (American coot)		12		12			
<u>Bucephala albeola</u> (buffle-head)		1		1			
<u>Bucephala clangula</u> (golden-eye)		1		1			
<u>Aythia affinis</u> (lesser scaup)			4	4			
<u>Melanitta deglandi</u> (white-winged scoter)			1	1			
<u>Pandion haliaetus</u> (osprey)		1	-	1			
<u>Corvus brachyrhynchos</u> (crow)			1	1			
Total	45	78	99	222	77.8	36.2	11.1
							33.3

Table 37

Extensivity of infection among gulls and mergansers, 1963-1965

No. examined	Ring-billed gull		California gull		Herring gull		Common merganser		Total		
	Adult	immat.	Adult	immat.	Adult	immat.	Adult	immat.	Adult	immat.	Overall
1963	18	2	-	-	14	3	6	2	38	7	45
1964	18	11	6	3	5	0	6	0	35	14	49
1965	19	40	7	4	12	6	1	0	39	50	89
Total	55	53	13	7	31	9	13	2	112	71	183
% infected											
1963	72.2	50.0	-	-	100	33.3	66.7	100	81.6	57.1	77.8
1964	72.2	27.3	83.3	33.3	80	-	16.7	-	65.7	28.6	57.1
1965	36.8	2.5	14.3	25.0	8.3	0	0	-	23.1	4.0	12.4
Total	60.0	9.4	46.2	28.6	61.3	11.1	38.5	100	57.1	14.1	40.4

Table 38
Monthly variations in extensity of infection - gulls and mergansers, 1963-1965

Month	No. examined			No. infctd		% infctd		Total Infctd	
	Total	Adult	Imm.	Adult	Imm.	Adult	Imm.	No.	%
April	4	4	0	2	0	50.0	0	2	50.0
May	3	1	2	1	0	100	0	1	33.3
June	6	1	5	0	0	0	0	0	0
July	71	37	34	16	2	43.2	5.9	18	25.4
August	43	24	19	9	7	37.5	36.8	16	37.2
September	35	28	7	25	1	89.3	14.3	26	74.3
October	21	17	4	11	0	52.4	0	11	52.4
Total	183	112	71	64	10	57.1	14.1	74	40.4

Table 39

Frequency of occurrence of gravid D. dendriticum and D. ditremum
in gulls and mergansers, 1963-1965.

Species of host	Number infctd.	Total no. of gravid worms	Number of	
			<u>D. dendriticum</u>	<u>D. ditremum</u>
<u>Larus delawarensis</u>	39	27 (19)*	11 (11)*	16 (14)*
<u>Larus argentatus</u>	20	13 (10)	5 (5)	8 (7)
<u>Larus californicus</u>	8	12 (6)	6 (5)	6 (4)
<u>Mergus merganser</u>	7	6 (4)	2 (2)	4 (2)
Total	74	58 (39)	24 (23)	34 (27)
Percentage of the total no. of gravid worms			41.0	59.0

* Number of host birds harbouring gravid worms.

Table 40

Monthly frequency of occurrence of gravid D. dendriticum and D. ditremum in gulls and mergansers, 1963-1965

Month	No. of birds examd	Total No. infected	Gravid worms		Gravid <u>D. dendriticum</u>		Gravid <u>D. ditremum</u>	
			No.	No. of birds	No.	No. of birds	No.	No. of birds
April	4	2	2	2	1	1	1	1
May	3	1	3	2	2	1	1	1
June	6	0	0	0	0	0	0	0
July	71	18	10	8	6	8.4	4	3
August	43	16	8	5	4	9.3	4	3
September	35	26	26	16	7	20.0	18	14
October	21	11	9	6	3	14.3	6	5
Total	183	74	58	39	24	12.6	34	27
								14.2

SUMMARY

The diphyllbothriid plerocercoids infecting certain species of fish in Kootenay Lake, B.C. and Iosegun Lake, Alberta, have been investigated. Seven species of plerocercoids were found in Kootenay Lake, and a single species in Iosegun Lake. Due to the sparsity of the useful literature on plerocercoids, and because identification of the species of this genus is almost exclusively based on characteristics of the adult worm, most of these plerocercoids could not be readily identified. Feeding experiments in which each type of plerocercoid was fed to individual hosts of four species were therefore conducted in order to obtain adult worms. By utilizing a combination of adult and plerocercoid characteristics, five of these plerocercoids were identified as D. dendriticum, D. ditremum, D. osmeri, D. cordiceps, and D. latum. The other two could not be identified to species.

Specific criteria in the genus Diphyllbothrium were evaluated before the species were described and compared with other species which are well characterized and fully described.

Comparative studies were carried out on the rate and pattern of growth of four species, namely, D. dendriticum, D. ditremum, D. osmeri, and D. cordiceps in two or more of the four host species used (dogs, cats, rats, and gulls), and observations were made on the time of maturity and apolysis, and the longevity of the worms in these hosts. No consistent rate of growth could be determined

for any of the species, the extent of variability in the length of worms reared simultaneously even in a single host being too great. But a pattern of growth common to all four species and which seemed to be correlated to some extent with the beginning of patency and apolysis, was observed.

A total of 1612 fish of 14 species and 9 families was examined for plerocercoids. Only five species from these families were infected. These were kokanee (Oncorhynchus nerka), rainbow trout (Salmo gairdneri), and dolly varden char (Salvelinus malma), from Kootenay Lake, and northern pike (Esox lucius) and walleye (Stizostedion vitreum) from Iosegun Lake.

D. ditremum was the most abundant species in Kootenay Lake salmonids, being found in relatively high intensities in practically 100% of kokanee, and in a high percentage of trout. All infected specimens of the three salmonids carried this species. Of the other species, only D. osmeri and D. dendriticum were relatively common, the latter only in trout. D. cordiceps was found only in trout encysted in the musculature or the viscera, or lying free in the latter location. D. latum, the only species found in Iosegun Lake, was found only twice in Kootenay trout. Only a small percentage (1%) of all plerocercoids recovered from all hosts, was in the muscle. The public health significance of these worms has been discussed, especially since two human infections, one definitely with D. latum, have been found in the

Kootenay Lake district during the study.

A positive correlation between the age and size of fish and the extensity and/or intensity of infection was noted and discussed. It was, however, difficult to ascertain the true extent of any seasonal variation of intensity in kokanee, due to the age and size composition of the samples examined at various times of year.

The pattern of infection in trout suggests that these fish acquire their infection through the agency of kokanee or some other small plankton-feeding fish. A similar route of infection, through a small fish, has been suggested from the data for dolly varden char, pike, and walleye as well.

In an attempt to discover the definitive hosts of these cestodes, 222 birds of 15 species, but mainly three species of gulls, as well as 13 mammals, were captured and autopsied (nearly all of these from the Kootenay Lake area). The mammals were not infected, but the gulls and one species of merganser were the only hosts found for D. dendriticum and D. ditremum. About 59% of the gravid worms recovered from these birds were D. ditremum, the remainder were D. dendriticum.

The data suggest that gulls acquire their infections of D. ditremum mainly by eating kokanee, and their D. dendriticum infections from the viscera of trout discarded on the lake and on open garbage dumps in the Kootenay Lake district.

The extensity of infection among adult birds was higher than among immatures (yearling mergansers and yearling and two-year old gulls). This might be due in part to the time of year when most of the latter were collected (spring and early summer).

A marked seasonal variation in the extensity of infection was observed, a higher extensity occurring in late summer and early fall when abundant infected kokanee, which have spawned and died, are readily available to potential hosts.

No definitive hosts have been found for the other species of parasites. The probability of a mammalian host especially in the life-cycle of D. osmeri, has, however, been suggested by the results of feeding experiments.

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